Volume 3, No. 2 | 2009

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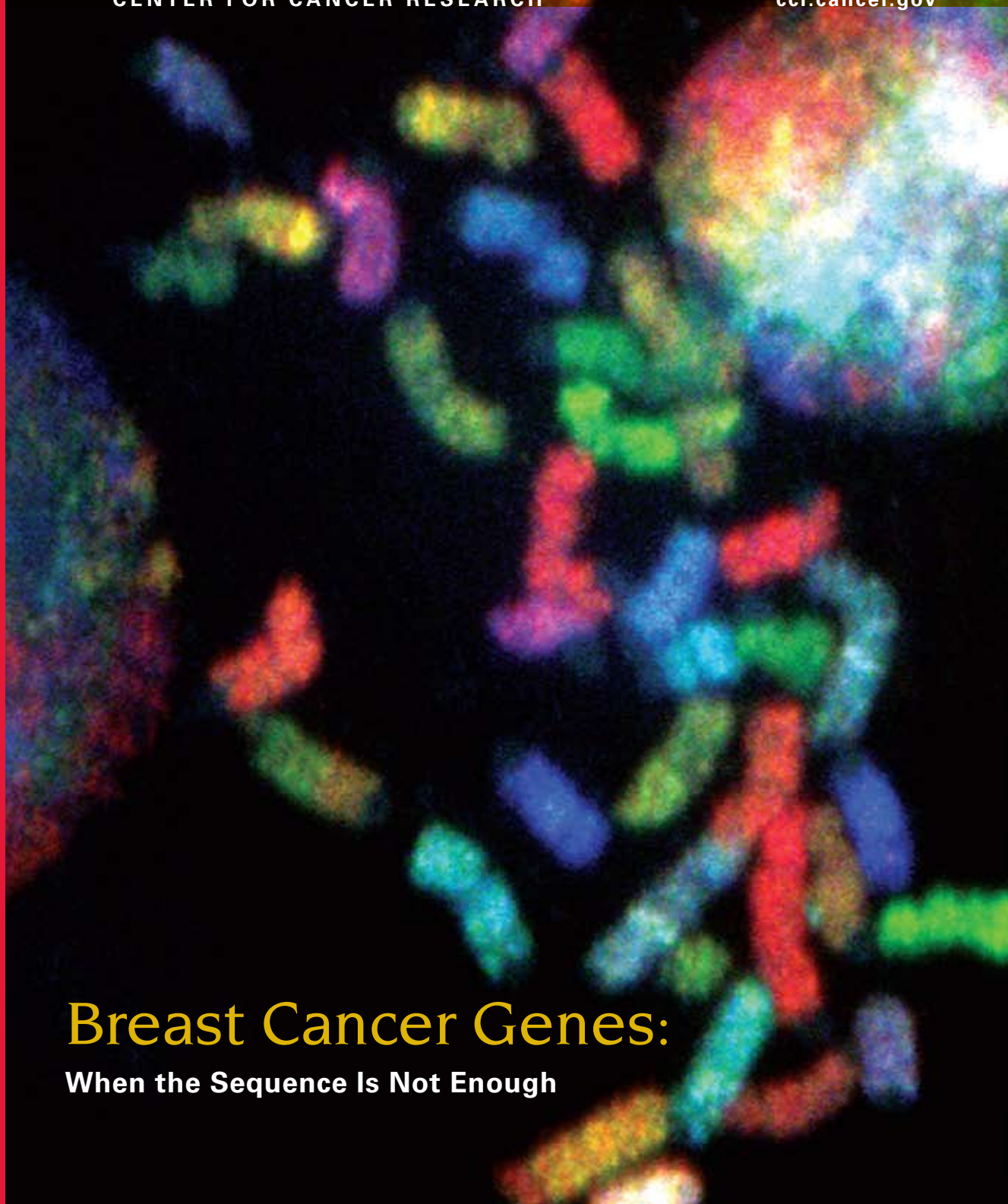

Breast Cancer Genes:

When the Sequence Is Not Enough

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

We invite your comments and suggestions about *CCR connections*.
Please email your feedback to tellccr@mail.nih.gov.

Center for Cancer Research

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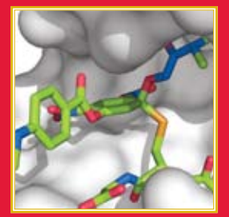

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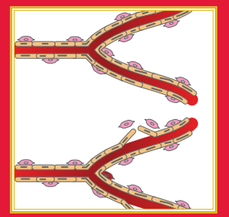

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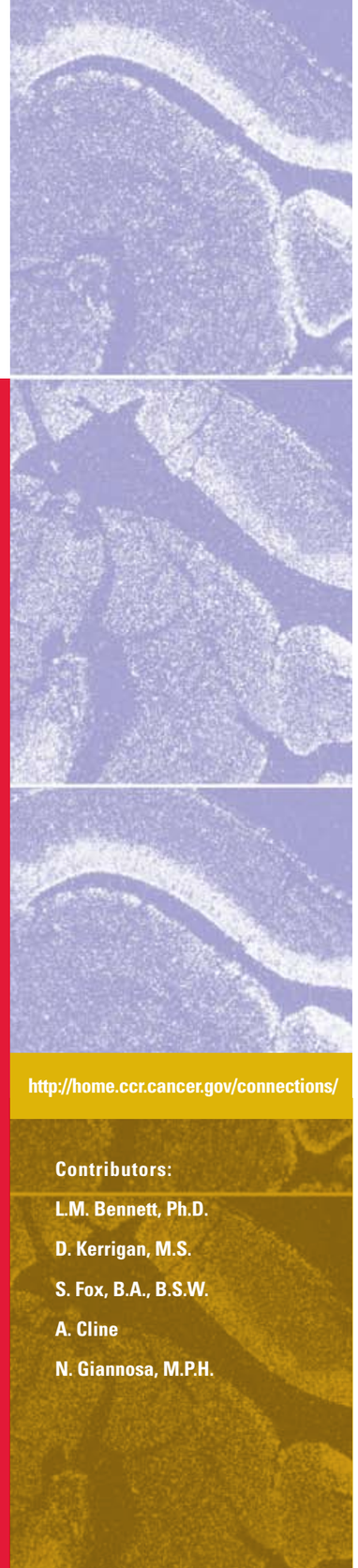
Breast Cancer Genes: When the Sequence Is Not Enough

CENTER FOR CANCER RESEARCH

The mission of CCR is:

To inform and empower the entire cancer research community by making breakthrough discoveries in basic and clinical cancer research and by developing them into novel therapeutic interventions for adults and children afflicted with cancer or infected with HIV.



http://home.ccr.cancer.gov/connections/

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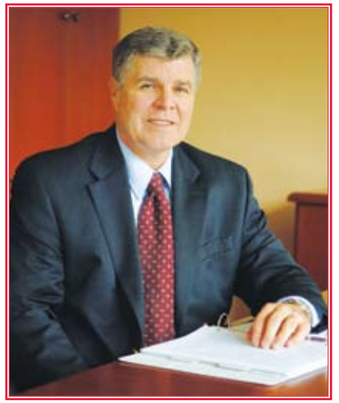
Changing Leadership, Unchanging Values

At the time of this writing, Francis S. Collins, M.D., Ph.D., has only recently been appointed the 16th Director of the National Institutes of Health (NIH). For any organization, the advent of a new leader is a time of great excitement, energy, and some uncertainty in the face of change, however necessary or welcome. In the days following his appointment, Dr. Collins has had the opportunity to articulate his vision for the NIH in multiple venues, and we are particularly pleased to see our efforts align so well with his stated priorities.



(Photo: B. Branson)

Robert H. Wiltrout, Ph.D.

Dr. Collins has outlined five key themes that characterize his vision for the NIH: 1) using high-throughput technologies to broaden the scope of our scientific questions; 2) translating new discoveries into diagnostic, preventive, and therapeutic strategies for disease; 3) putting science to work for health care reform; 4) increasing emphasis on global health; and 5) reinvigorating biomedicine with new and stable funding that rewards risk as well as scientific rigor alongside high-quality training that builds the next generation of scientists.

In this issue of *CCR connections*, you will find several examples of CCR researchers and clinicians actively engaged in advancing this ambitious agenda. In "Big Things in Small Packages: Small RNAs Play a Big Role in Cancer Biology" and "Brain Cancers: Not All Made the Same," we see two examples of CCR clinical scientists applying comprehensive gene expression analyses to uncover biomarkers in liver and brain cancers, respectively, that will one day help to inform the choice of treatment. In "Cut to the Cure," Staff Clinician Marybeth Hughes, M.D., tells us about the daily mix of research and patient care that is part of her overall ambition to translate discovery into treatment and use treatment to inform discovery.

The CCR leadership is also seeking more effective ways for our scientists to partner with industry to translate their research into treatments. In "Partners in Science: The Umbrella CRADA Streamlines Collaborations Between CCR and Industry," we learn about a collaborative agreement between CCR investigators and AstraZeneca that will serve as a model for future engagements with industry. As our former CCR colleagues Nancy Jenkins, Ph.D., and Neal Copeland, Ph.D., note in their commentary "Science in Singapore: Aiming High for Biomedical Research," research institutes like ours and pharmaceutical companies should be natural scientific allies—the science needed to support successful drug development is too complex to go it alone.

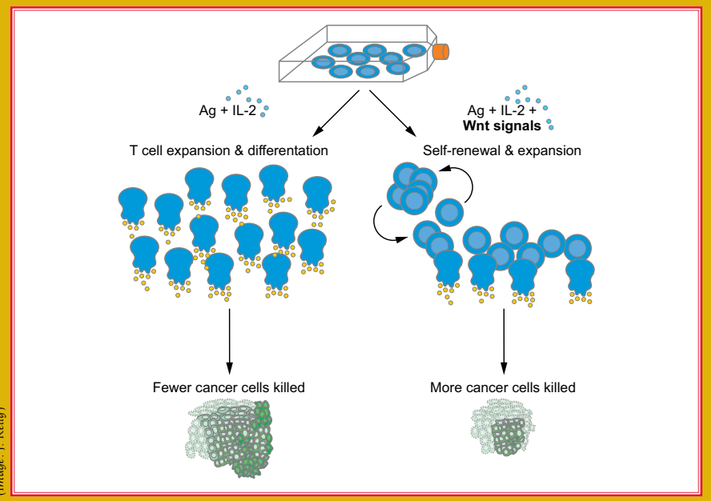
We introduce a new series in this issue—In Conversation—in which we will be talking with some of our CCR fellows to learn about their experiences and aspirations. An important part of our mission is to train the next generation of scientific leaders, and we want to inspire other young people to invest their talents in biomedical research. Of course, as Dr. Collins has emphasized, for science to flourish and inspire the next generation to service, its leadership must provide the means to support that service. In "Breast Cancer Genes: When the Sequence Is Not Enough," we follow the 10-year journey of CCR Investigator Shyam Sharan, Ph.D., which has led to innovative tools to tackle the persistent mysteries of breast cancer.

We look forward to working with Dr. Collins over the coming years to further our mission: To inform and empower the Nation's research community by making breakthrough discoveries in basic and clinical research and by developing them into novel therapeutic interventions for adults and children with cancer or HIV infection. The scientific strategies to fulfill this mission will no doubt change over time, but the goal remains the same—to prevent, cure, or make cancer a manageable, chronic disease.

A Cure for the Incurable?

Using the Body's Immune System to Treat Metastatic Cancers

The long fight against cancer has rewarded us with many treatments—chemotherapy, radiotherapy, and surgery—that have improved the prognoses for many types of cancer. Cures for metastatic disease have thus far been less forthcoming; however, recent advances in cancer immunotherapy may lead us in a promising new direction.



(Image: J. Kelly)

Stimulating antitumor T cells in the presence of drugs that mimicked Wnt signaling suppressed the process of T-cell differentiation, so that the lymphocytes remained in a stem-like state. These T cells could self-renew and differentiate into various CD8+ memory and killer T cell subsets. When adoptively transferred into a tumor-bearing host, they could kill more tumor cells than non-Wnt-stimulated T cells.

Although the concept of immunotherapy for cancer has been around for over a century, its history has seen alternating cycles of optimism and frustration. Cancer vaccines have thus far proven insufficient by themselves to induce cancer regression reliably. Adoptive immunotherapies in which a patient's immunological components (such as T lymphocytes) are isolated, modified *in vitro*, and then re-infused back into the patient could, in principle, contribute to therapeutic progress. In the July 2009 issue of *Nature Medicine*, Nicholas Restifo, M.D., Senior Investigator in the Tumor Immunology Section at CCR, introduced the adoptive transfer of genetically engineered tumor-specific T memory stem cells (T_{SCM}) as an exciting new strategy towards an effective cancer therapy.

Previous experiments using the adoptive transfer of naturally occurring T lymphocytes have given variable results. The transplanted cells do not always induce optimal anti-tumor responses, and as they are already terminally differentiated into $CD8^+$ killer T cells, their therapeutic effects are relatively short-lived. Restifo and colleagues wanted to limit the differentiation so as to maximize proliferation *in vivo* after transfer, and they did this by pharmacologically generating stem-like T cells with an enhanced ability to renew themselves and proliferate—qualities most associated with anti-tumor effectiveness.

Using a mouse model, the researchers isolated young T lymphocytes and, *in vitro*, stimulated anti-tumor T cells in the presence of drugs that mimicked Wnt signaling. Wnts control developmental programs that are important for embryogenesis and development, including T lymphocyte development. The researchers found that Wnt signaling plays a key role in the maintenance of "stemness" in mature $CD8^+$ memory T cells. Stimulating Wnt signaling suppressed the process of T cell differentiation so that the lymphocytes remained in a young, stem-like state with a high proliferative potential. This new class of T_{SCM} cells could self-renew and differentiate into various $CD8^+$ memory and killer T cell subsets following adoptive transfer back into the tumor-bearing mice.

The ability to pharmacologically induce T_{SCM} cells has considerable implications for adoptive immunotherapies and the design of new vaccine strategies. Because of their increased proliferative responses, enhanced survival capacity, and superior anti-tumor activity, only a small number of T_{SCM} cells, together with a recombinant cancer vaccine and interleukin-2, were sufficient to trigger the destruction of large tumors in mice.

"Using the immune system to cause rejection of cancer was once considered a radically alternative, futuristic approach," said Dr. Restifo. "But the adoptive transfer of young T cells derived from naturally occuring or genetically-engineered tumor-specific cells is a reality. For some patients with metastatic cancer, imunotherapies based on the adoptive transfer of T lymphocytes can be curative."

To learn more about Dr. Restifo's research, please visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?profileid=5762.

Big Things in Small Packages:

Small RNAs Play a Big Role in Cancer Biology

Exquisitely tuned gene expression is essential to orchestrate both the development and functioning of the myriad cell types in the human body. When that tuning goes awry, one result is cancer. Small microRNAs (miRNAs) have emerged relatively recently as key modulators of gene expression, acting at a stage between transcription of the genes and translation into proteins. Although they are tiny, miRNAs—at a little over 20 nucleotides long—pack a big punch since each regulates a variety of genes, and they are involved in diverse pathological processes, including cell proliferation and death.

Of course, miRNAs are themselves tightly controlled. Faulty regulation of miRNAs, especially downregulation of these minuscule molecules, has been found in every tumor type tested thus far and has also been implicated in cancer progression and metastasis. Recognizing this, Xin Wei Wang, Ph.D., Head of the Liver Carcinogenesis Section in CCR's Laboratory of Human Carcinogenesis, asked whether miRNAs could serve as biomarkers for liver cancer. "Hepatocellular carcinoma, which makes up about 90 percent of liver cancer, is very heterogeneous in terms of biology and clinical outcome," explained Dr. Wang. "Our hope is to identify biomarkers that will distinguish the patients who will benefit from different treatments."

Knowing that hepatocellular carcinoma (HCC) has a two- to six-fold higher incidence in men than in women, Dr. Wang and his collaborators hypothesized that the differences in tumor microenvironments between the genders may be associated with prognosis. Therefore, they examined cancerous and noncancerous liver tissues in men and women to discover the biological and genetic differences that could be attributed to HCC development and progression.

In the October 2009 issue of *The New England Journal of Medicine*, the researchers reported a correlation between one miRNA, miR-26, and survival and response to interferon-α treatment in male and female liver cancer patients. The team analyzed three independent cohorts and found that miR-26 expression levels were higher in nontumor liver tissue from female patients than from male patients,

(Image: Ethan Tyler)

A patient's microRNA profile of low miR-26 *(represented by the yellow miRNA)* could be used to predict effectiveness of interferon therapy *(vials)* for hepatocellular carcinoma.

and liver tumor tissue had reduced miR-26 expression when compared to normal liver tissue, indicating that miR-26 is a liver tumor suppressor. The reduced miR-26 expression was strongly associated with particular patterns of overall gene expression, including activation of the NFκB/IL-6 signaling pathway. Because estrogens inhibit IL-6 expression, Dr. Wang and his colleagues suggest that this pathway may contribute to the sex disparity in development of this tumor type. The researchers also showed that patients with reduced miR-26 expression in their tumors had lower survival rates but were more responsive to interferon-α treatment than patients with normal miR-26 expression.

Dr. Wang looks to these results as a clinically useful tool that could ultimately turn miRNA profiling into a standard procedure for liver cancer patients. Having a genetic profile that can stratify patients would allow clinicians to decide on an appropriate course of treatment early after diagnosis that is as individualized as each tumor and each patient.

To learn more about Dr. Wang's research, please visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?profileid=5764.

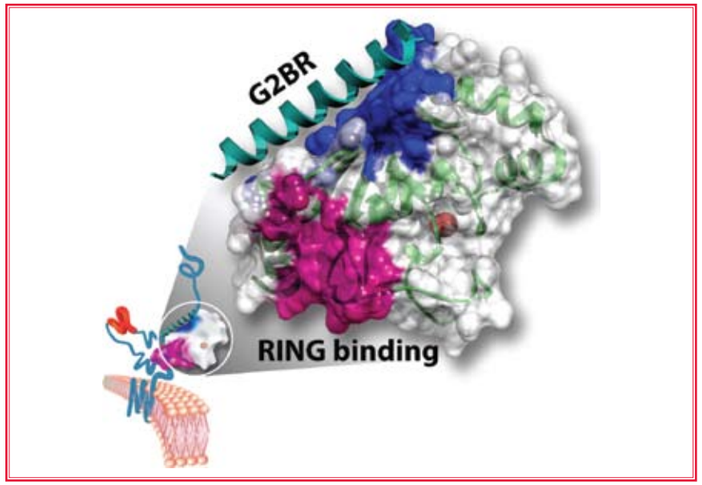
Targeted Destruction:

Novel Interactions in Ubiquitylation and Tumorigenesis

The controlled destruction of proteins is as important as their synthesis for maintaining cell integrity. A process called ubiquitylation tags proteins for degradation and plays a crucial part in cell cycle regulation, DNA repair, cell growth, and immune function, among other processes. Its dysfunction contributes to pathogenesis, including the development of cancer.

Ubiquitylation is a multistep process in which a ubiquitin tag passes through an assembly line of enzymes—E1, E2, E3—before being attached to a protein. E1 activates the ubiquitin molecule at the start of the process, and E3s (also known as ubiquitin ligases), of which there are more than 500 in mammals, are ultimately responsible for directing the ubiquitin tag to specific protein targets. E2s sit in the middle, coordinating and potentially regulating their fellow enzymes through specific binding sites. In the June 26, 2009 issue of *Molecular Cell*, CCR researchers involved in a multidisciplinary collaboration have published one key to the mechanism of action of a specific E3 family member, gp78, which controls the levels of a metastasis suppressor.

An earlier study by Allan Weissman, M.D., Chief of the Laboratory of Protein Dynamics and Signaling at CCR, showed that high levels of gp78 promote the spread of cancer by tagging a protein for degradation that suppresses metastasis and that the E3 activity of gp78 was required for this degradation to occur. Weissman's lab also demonstrated that, in addition to a known interaction site for E2 enzymes (the RING finger domain), gp78 has a unique region called the G2BR that strongly binds to its corresponding E2.



(*Image: A. Kane, Scientific Publications, NCI-Frederick*)

Model *(bottom left)* of the ubiquitin ligase gp78 interacting with its cognate E2 in the endoplasmic reticulum membrane. Structural details of the interactions of the E2 with G2BR *(helix represented in cyan)* and RING finger domains *(magenta binding surface)* of gp78 are revealed by NMR and X-ray structural studies.

In this new work, Andrew Byrd, Ph.D., Head of the Macromolecular NMR Section at CCR; Xinjua Ji, Ph.D., Head of the Biomolecular Structure Section at CCR; Dr. Weissman; and their colleagues used advanced structural techniques to study the interaction between gp78 and its E2 and uncovered a previously unknown mechanism by which ubiquitylation can be regulated. Whereas previous work identified the interaction site of RING finger domains on E2 enzymes, the researchers found that the gp78 G2BR binds to an additional distinct area of E2. This G2BR binding causes conformational changes to the E2 that allow the gp78 RING finger domain and the E2 to bind 50 times more tightly than they would otherwise. "This is the first demonstration of an allosteric mechanism whereby interactions with a RING finger domain are enhanced by binding to a second discrete domain within the ligase," said Dr. Byrd. "This represents a significant shift in the existing paradigm for E1-E2-E3 function."

Further research showed that this increased binding strength enhances ubiquitylation of target proteins by gp78, so blocking G2BR function would inhibit degradation of the proteins that suppress cancer metastasis. This team is currently collaborating with other CCR scientists to further define the interactions of E2s and RING finger domains and to design and construct potential inhibitors of gp78 for testing in animal models. With successes *in vivo*, the team ultimately hopes to add ubiquitylation-regulating agents to the armamentarium of cancer drugs.

To learn more about Dr. Byrd's *research, please visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?profileid=5544.*

To learn more about Dr. Weissman's *research, please visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?profileid=6524.*

To learn more about Dr. Ji's *research, please visit http://ccr.cancer.gov/staff/staff.asp?profileid=5860.*

Recent CCR Awards

2009 Federal Laboratory Consortium Mid-Atlantic Laboratory Director of the Year Award

For outstanding contributions in support of technology transfer

Robert H. Wiltrout, *Ph.D.*
Director, Center for Cancer Research

(*Image: S. Kaushan*)

Robert H. Wiltrout, Ph.D., Director of CCR, receives the 2009 Federal Laboratory Director of the Year Award, Mid-Atlantic Region

American Society of Hematology 2009 Dameshek Prize

For landmark contributions to the diagnosis and treatment of lymphomas

Louis M. Staudt, *M.D., Ph.D.*
Metabolism Branch

Inducted to American Academy of Arts and Sciences

Sankar Adhya, *Ph.D.*
Laboratory of Molecular Biology

Association of Military Surgeons of the United States Andrew Craigie Award

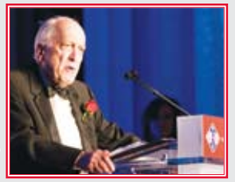
For outstanding advancement of pharmacy within the federal government

William Douglas Figg, Sr., *Pharm.D.*
Medical Oncology Branch

Annual European Association of Nuclear Medicine Springer Prize for Best Basic Science Paper

For [^{18}F]FBEM-$Z_{HER2:342}$–Affibody molecule—a new molecular tracer for in vivo monitoring of HER2 expression by positron emission tomography

Gabriela Kramer-Marek, *Ph.D.*
Sang Bong Lee, *Ph.D.*
and **Jacek Capala, *Ph.D.***
Radiation Oncology Branch



(*Image: S. Howe*)

Thomas Alexander Waldmann, M.D., receives a 2009 *Career Achievement Medal* from the Partnership for Public Service at a gala in Washington, D.C. on September 23, 2009.

Career Achievement Medal

Thomas Alexander Waldmann, M.D., Chief of the Metabolism Branch at CCR, received a 2009 *Career Achievement Medal* for his exemplary innovation and accomplishments in medical research.

Over the course of five decades, Waldmann has made significant advances in the treatment of patients with cancer, AIDS, and multiple sclerosis, including the development of new therapies for previously fatal forms of T cell leukemia and Hodgkin's lymphoma.

The major focus of Waldmann's work has been in the field of cytokines, molecules that control human immune responses. He was intimately involved in the development of the groundbreaking medicine Zenapax®, which produces complete remission in over 60 percent of patients with Hodgkin's lymphoma who are unresponsive to any other treatment.

This Achievement Medal is one of nine *Service to America Medals* awarded by Congressional leaders and the Partnership for Public Service, a nonprofit, nonpartisan organization that works to revitalize the federal government. This year's recipients were selected from a group of over 400 nominees drawn from almost every major government agency.

International Antonio Feltrinelli Prize for Medicine

Ira Pastan, M.D., Chief of the Laboratory of Molecular Biology at CCR, received the 2009 international *Antonio Feltrinelli Prize for Medicine* for his lifetime work unraveling surface cell receptors and for developing effective immunotoxin therapies to treat both rare and common cancers—an accomplishment requiring 20 years of persistent pursuit.

Early immunotoxins based on the bacterial protein *Pseudomonas exotoxin A* (PE) required expensive, complex chemistry to link the lethal toxin to antibodies that could target tumor cells. Using recombinant DNA techniques and his extensive knowledge of cell surface receptors, Pastan has engineered smaller, more nimble immunotoxins to target several different tumor types. That shift from chemistry to genetic engineering for producing custom immunotoxins has been a major breakthrough.

Awarded every five years, the *Antonio Feltrinelli Prize for Medicine* includes a valuable gold medal and a sizable monetary prize awarded by the Accademia Nazionale del Lincei. The organization, founded in 1603, includes Galileo as a member and is Italy's most prestigious scientific society.

Keeping Cell Suicide in Check

For most of human history, and even now, we have treated the sick with medicines whose mechanisms of action are a mystery. One such case is TNF-related apoptosis-inducing ligand (TRAIL), a protein we produce naturally as a regulator of programmed cell death (apoptosis) that is also administered as an effective cancer therapy. A better understanding of how TRAIL activates apoptosis preferentially in cancer cells could lead to further improvements in cancer therapies. To that end, Stéphanie Solier, Pharm.D., Ph.D., a Postdoctoral Fellow in the Laboratory of Molecular Pharmacology at CCR, and Yves Pommier, M.D., Ph.D., who heads the lab, describe new insights into TRAIL's mechanism of action in the January 2009 issue of Molecular and Cellular Biology.

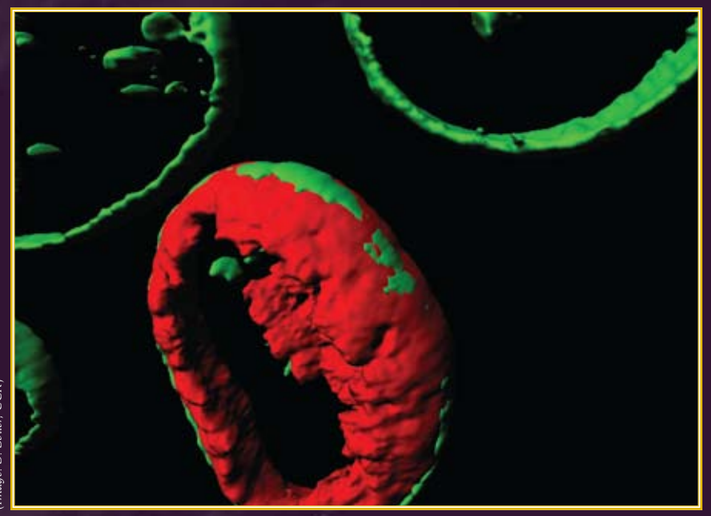
In normal cells, apoptosis can be activated in two ways: by direct damage within the cell or by an external pathway that is activated when certain signaling proteins bind to the cell's surface receptors and engage a death-inducing signaling complex. Although we know that TRAIL activates the latter system, the researchers proposed that TRAIL could also be utilizing components of the DNA damage response (DDR) pathway to destroy cancer cells. To test their hypothesis, they monitored the effects of TRAIL on specific proteins in cultured cancer cells. Two key findings emerged.

TRAIL was found to activate Chk2, a protein involved in the regulation of checkpoints used by cells to determine whether DNA damage is serious enough to proceed with programmed cell death. Drs. Solier and Pommier showed that activation of Chk2 amplifies apoptotic signaling and approximately doubles the number of dying cells that otherwise survive TRAIL treatment.

Whether it leads to apoptosis or not, DNA damage also induces modifications to histone proteins—key regulators of DNA structure and function—including phosphorylation of the histone H2AX (γ-H2AX) that can be visualized using a specific antibody developed by William Bonner, Ph.D., in the Laboratory of Molecular Pharmacology at CCR. Whereas DNA damage activates γ-H2AX locally in the nucleus, TRAIL induces an initial ring of γ-H2AX along the entire periphery of the nucleus that precedes apoptotic nuclear fragmentation.

In the June 2009 issue of *Cell Cycle*, Drs. Pommier and Solier followed up on this intriguing difference in histone modifications, proposing a previously unrecognized histone phosphorylation signature for apoptosis and demonstrating how this signature, together with the γ-H2AX ring, provides a new feature to monitor and study cell death. "The NCI recognized very early the value of γ-H2AX, and it has turned into an increasingly clinically useful biomarker," said Dr. Pommier.

These findings have many clinical implications for the treatment of cancer. The discovery that γ-H2AX activation by TRAIL uses segments of the DDR pathway provides a rationale for combining TRAIL and DNA-damage agents for anticancer therapy. It may also be possible to predict the effectiveness of TRAIL therapy based on the level of Chk2 in tumors, and activation of Chk2 in precancerous tumors may be able to prevent or delay cancer development.



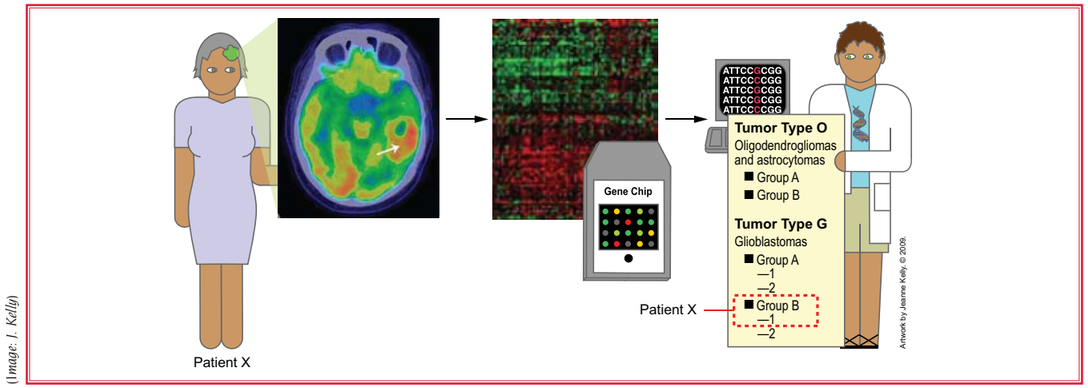
(Image: S. Solier, CCR)

A representative 3-dimensional image of peripheral nuclear gamma-H2AX distribution in response to TRAIL *(gamma-H2AX is red and the nuclear envelope is green).*

To learn more about Dr. Pommier's research, please visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?profileid=5812.

Brain Cancers: Not All Made the Same

Primary brain tumors are an increasingly significant cause of cancer-related mortality in the United States. Gliomas, in particular, have attracted more attention in recent decades because there are several reports suggesting an increasing trend in incidence rates, and the prognosis for patients with high-grade gliomas is still very poor. Of the approximately 17,000 Americans diagnosed each year with malignant gliomas, about 50 percent survive one year after diagnosis, and 25 percent survive after two years. Because the disease is nonetheless relatively rare, progress in understanding the disease has been slow. Oftentimes, two glioma patients whose tumors look the same in an MRI or under a microscope experience completely different outcomes despite being given the exact same treatment. In fact, these seemingly alike tumors actually have distinct genomic profiles and molecular abnormalities. But from standard pathology, we could never discern the difference.



(Image: J. Kelly)

Howard Fine, M.D., and colleagues analyzed genomic profiles from patients diagnosed with malignant glial cells using computer-generated groupings to subclassify the brain tumors into two major groups: O type tumors, which were predominantly oligodendrogliomas and astrocytomas, and G type tumors, which were mostly glioblastomas. They further divided the G tumors into two additional levels of subclassification.

"To understand how best to treat our patients, we really have to get past the 200-year-old tradition of just looking at a microscope and saying, 'Oh it kind of looks like that tumor, or it kind of looks like this tumor,'" said Howard Fine, M.D., Chief of the Neuro-Oncology Branch at CCR. To that end, Dr. Fine and colleagues undertook a large-scale analysis to classify different types of glioblastomas based on the simultaneous expression of genes within the tumor cells. Their findings were published in the March 2009 issue of *Cancer Research*.

In the first gene expression profiling study to look at the whole family of gliomas rather than just a subset of gliomas, Dr. Fine and colleagues used a series of statistical and mathematical models to analyze the results from arrays containing 20,000 to 25,000 genes at one time. The researchers were able to identify two major types of gliomas: an oligodendroglioma-rich group, further divided into two subgroups, and a glioblastoma-rich group, divided into four subgroups. These subgroups have many of the same molecular pathways and genetic mutations, so they are more biologically similar and, therefore, should represent patient groups that are more likely to respond to similar treatments.

These subgroups have been correlated with histological and clinical features, but they still need to be validated by data from more patients to determine the extent to which they are meaningful for diagnosis and prognosis. The researchers will need to determine if their analysis method helps to select therapies that are more effective for patients in that particular subgroup "For me," said Dr. Fine, "as a translational scientist, and first and foremost a physician who's taken care of 20,000 patients with gliomas in the past 20 years, what I really care about is: Will the subgroups allow us to improve the outcomes of our patients? That's ultimately the only validation that really matters."

To learn more about Dr. Fine's research, please visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?profileid=5635.

The NIH Pediatric and Wildtype GIST Clinic

Pediatric gastrointestinal stromal tumor (GIST) is a rare disorder affecting less than 200 patients in the United States. Because this disease is so rare and the biological differences between children and adults affected with GIST are so great, it has been difficult to study this disorder and determine the best therapy for pediatric patients. In an effort to advance research on GIST, Su Young Kim, M.D., Ph.D., and Lee J. Helman, M.D., in the Pediatric Oncology Branch at CCR, and Constantine A. Stratakis, M.D., D.Med.Sci., in the National Institute of Child Health and Human Development, have led the development of the Pediatric and Wildtype GIST Clinic.

The GIST Clinic is a collaborative effort between clinicians, research scientists, and advocates across the nation to better understand the pathogenesis of GIST, to develop innovative national clinical trials, and to assess the best treatment approaches for these patients. Dr. Kim also initiated a "virtual" GIST clinic, a secure NCI-based Web site (www.pediatricgist.cancer.gov) that will store medical information for patients, physicians, and researchers. The goal is to create a database of all young patients with GIST around the world that contains information on their clinical history, responses to prior treatments, histopathologic results, radiographic assessments, and genetic/molecular analyses in order to find a cure for this rare disease collaboratively.

(Photo: S.Y. Kim, CCR)

Four mothers and their daughters await appointments at the Pediatric GIST clinic.

To learn more about Dr. Kim's research, please visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?profileid=7870.

In Conversation:

Research Fellow Aaron Schetter, Ph.D., M.P.H.

(Photo: R. Baer)

Aaron Schetter, Ph.D., M.P.H.

CCR: Aaron, you have been a Fellow at CCR for four years now—what brought you to the field of cancer research?

Aaron: I did my Ph.D. at Cornell, in Ken Kemphues' lab, studying the genes involved in early development of the model organism *C. elegans*. I was fortunate to arrive just in time to take advantage of RNAi as a powerful new tool for genetic screening.

Then, while I was in graduate school, I was actually diagnosed with Hodgkin's lymphoma. In terms of cancers, if you have to get one, that's not a bad one to get—it is usually curable, although the chemotherapy makes you miserable for a few months. After that, I decided that I wanted to change the direction of my research to something that is more relevant to cancer.

CCR: Wow, what a powerful and personal motivation for your career. How did you decide where to pursue it?

Aaron: I applied for an NCI Cancer Prevention Fellowship. The fellowship takes people from a broad set of backgrounds and provides an opportunity to go back to school and earn a Master's in Public Health (M.P.H.). It then sponsors postdoctoral research at NCI for three additional years. Once I had my master's, the choice for me came down to picking a laboratory that was investigating therapeutic targets or biomarkers for cancer.

What interested me about Curt Harris's lab was that he was studying miRNAs, which I thought had high potential to be developed into biomarkers and therapeutic targets. Their small size made them easy to detect, knock down, or overexpress. At the time, one of the postdocs in the lab, Nozomu Yanaihara, had a project in which he found miRNAs that could predict survival in lung cancer.

I thought it would be great to do something similar for colon cancer.

CCR: And, have you succeeded in finding a biomarker for colon cancer?

Aaron: In fact, we have. We published a paper in *The Journal of the American Medical Association* last year that was the first to take miRNA expression profiles and predict survival and therapeutic outcome in colon cancer.

We are also following up on one of the most significant miRNAs from that biomarker study—mir-21—to see if it could be a useful therapeutic target. Because mir-21 was upregulated in patients that did not respond as well to chemotherapy, we are trying to sensitize colon cancer cells in culture to chemotherapy by knocking down mir-21. All of this is far away from the clinic because we don't have great ways to affect miRNAs in people. But, it sets us on the path.

CCR: Where do you see yourself in five years?

Aaron: I would like to take the kind of work I do now into the pharmaceutical industry to identify and potentially develop new drug targets. I want to work on a project that could end up treating disease.

CCR: And what advice would you give graduate students interested in coming to CCR?

Aaron: The biggest thing is to look for an environment and a group of people that match your research interests. Overall, I think the great thing about being here is that you are surrounded by floors of people working on cancer. The knowledge base about cancer even in this building alone is larger than you could find at many other institutions. Through journal clubs, meetings, and seminars, you are exposed to all kinds of insights and technologies and just have to walk down the hall to find out more.

Staff News at CCR

new tenure-track scientists

(Photo: M. Spencer)

Electron Kebebew, M.D.

Kebebew joins CCR's Surgery Branch. He received his M.D., surgical training, and completed an NCI surgical oncology fellowship at the University of California San Francisco (UCSF). He was an Associate Professor at UCSF before joining the Surgery Branch as a Senior Investigator in July 2009. His research focuses on molecular changes in endocrine carcinogenesis with the goal of identifying diagnostic markers and targets for therapy.

(Photo: M. Spencer)

Udo Rudloff, M.D.

Rudloff joins CCR's Surgery Branch. He received his M.D. from Heidelberg University School of Medicine, Germany. He completed his general surgery residency at the New York University School of Medicine and his fellowship in surgical oncology at Memorial Sloan-Kettering Cancer Center. Rudloff's research is focused on studies of genetic abnormalities in cancer and the development of personalized, molecular therapies targeted to these genetic alterations and the individual genetic fingerprint of patients' tumors. His clinical interests are in the management of malignancies of the liver, pancreas, and gastrointestinal tract.

announcement

(Photo: R. Baer)

William L. Dahut, M.D.

Dahut has been appointed Clinical Director of CCR. He received his M.D. from Georgetown University in Washington, D.C. and completed clinical training in internal medicine at the National Naval Medical Center in Bethesda, Md., followed by training in hematology and medical oncology at the Bethesda Naval Hospital and the Medicine Branch of NCI. Dahut worked as an attending physician in the NCI-Navy Medical Oncology Branch until 1995. He then joined the faculty of the Lombardi Cancer Center at Georgetown University before returning to the former NCI Medicine Branch in 1998 as Head of the Prostate Cancer Clinic. In 2002, he became Chief of the GU/GYN Clinical Research Section in the Medical Oncology Clinical Research Unit. His primary research interest has been in the development of novel therapeutic strategies for the treatment of adenocarcinoma of the prostate.

newly tenured CCR scientists

Michael Emmert-Buck, M.D., Ph.D.
Laboratory of Pathology

Pengnian Charles Lin, Ph.D.
Mouse Cancer Genetics Program

Shyam Sharan, Ph.D.
Mouse Cancer Genetics Program

Zhi-Ming Zheng, M.D., Ph.D.
HIV and AIDS Malignancy Branch

Brigitte C. Widemann, M.D.
Pediatric Oncology Branch

Victor B. Zhurkin, Ph.D.
Laboratory of Cell Biology

Alan M. Krensky, M.D.
Laboratory of Cellular and Molecular Biology

S. Perwez Hussain, Ph.D.
Laboratory of Human Carcinogenesis

Partners in Science:

The Umbrella CRADA Streamlines Collaborations Between CCR and Industry

Researchers across CCR are studying the molecular mechanisms that underlie multiple diseases. Some take as their starting point the mutations that confer genetic susceptibility in familial cancers; others may start with animal models. But the goal is the same–to prevent and treat disease by manipulating dysfunctional molecular networks. Although there are many research tools available to tease apart these networks, ultimately what is required are drugs that can be administered safely and effectively to patients. The pharmaceutical industry has a number of compounds in pipelines that are usually narrowly focused on a few target diseases. CCR has the expertise to test these compounds—alone or in combination—in robust preclinical models, as well as in the clinic. The advantages of collaboration are clear, but historically it has been difficult for individual investigators to broker the necessary negotiations with individual companies. CCR's Office of Policy and Intellectual Property, led by Eric Hale, J.D., M.B.A., has recently entered into a new kind of agreement between CCR and AstraZeneca—an Umbrella CRADA—which has made this type of collaboration much easier.

(Photo: R. Baer)

Eric Hale, J.D., M.B.A. *(left),* Grace Chao Yeh, Ph.D. *(center),* and Li Guo, Ph.D. *(right),* work in CCR's Office of Policy and Intellectual Property to adapt the Cooperative Research and Development Agreements (CRADAs) to benefit both the scientists at CCR and the companies that wish to collaborate with them.

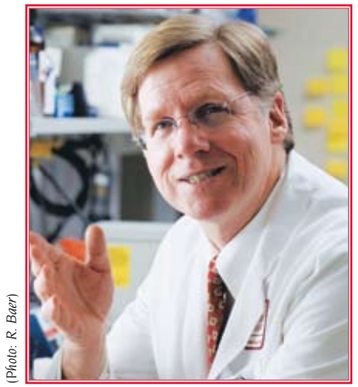
Beverly Mock, Ph.D., Head of the Cancer Genetics Section of CCR's Laboratory of Cancer Biology and Genetics, is eager to test AstraZeneca's inhibitor of mTOR (mammalian Target of rapamycin) in her xenograft models of multiple myeloma. In her research, she and her colleagues have recently demonstrated that a combination of the mTOR inhibitor rapamycin and an inhibitor of histone deacetylase (HDAC) has a powerful antitumor effect. Their analysis of the signaling pathways involved leads them to believe that the AstraZeneca compound may produce a similar effect, and validation of this hypothesis will be additional evidence towards their model of abnormal signaling at the root of plasma cell tumors.

W. Marston Linehan, M.D., Chief of CCR's Urologic Oncology Branch, has already started using the AstraZeneca mTOR inhibitor in cell lines derived from a patient with Birt-Hogg-Dube (BHD) syndrome and has seen very promising results. The inhibitor could be taken into clinical trials if the drug reaches that stage of development. Independently, their research on the mechanisms of action in this hereditary kidney cancer has converged with the targets of AstraZeneca's drug in what Linehan hopes will be "a perfect match."

Through the Umbrella CRADA mechanism, CCR is now offering

(Photo: B. Branson)

Beverly Mock, Ph.D.



(Photo: R. Baer)

W. Marston Linehan, M.D.

companies the means to study the biological activities of compounds in the context of highly developed scientific and clinical research programs that confer the ability to ask questions that companies are not themselves equipped to study. "These signals are important in validating internally that what you have spent a lot of time and treasure developing actually has biological activity," explained Gregory Curt, M.D., AstraZeneca's U.S. Medical Science Lead.

It is not by chance that one of the most successful Umbrella CRADAs was negotiated between CCR and AstraZeneca. Curt, the man responsible for establishing the collaboration on behalf of the company, spent 22 years at NCI, including 12 years as Clinical Director of CCR. "I know the program intimately and recognize what it can do uniquely," explained Curt. "We [pharmaceutical companies] tend to look at drugs from the prism of drugs. NCI tends to look at drugs from the prism of diseases. There is a real partnership to be gained there."

Why an Umbrella CRADA?

A standard CRADA is a written agreement between a federal research organization and one or more federal or non-federal parties (collaborators) to work together as partners on a research project of mutual interest (see "CRADA History"). Until recently, CRADAs at NCI have all been handled on an individual basis. An investigator conceives an idea (which he or she would discuss with a company representative), develops a research plan, and works with NCI's Technology Transfer Center to develop the proposed agreement. This agreement is subject to review by an NIH-wide CRADA subcommittee to ensure that issues such as fair access and conflicts of interest are handled appropriately. This process can take up to a year and can create inconsistencies even across different CRADAs with the same company.

"I am not a lawyer, nor a technology transfer guru," explained Linehan, although he nonetheless sometimes found himself sitting down with companies to allay misplaced fears of government obstacles to collaboration with industry. "Once you have that out of the way, it's fine," he added, noting that when all you want to do is make progress scientifically, there are far fewer barriers than most companies imagine. "But the process takes a long time, and if you are trying to do this for each company, it slows you down and slows you down, and you just give up after a while."

To streamline the CRADA process for all parties concerned, Hale and his colleagues Grace Yeh, Ph.D., and Li Guo have taken it to a new level. A first of its kind for the federal government or industry, the Umbrella CRADA is an agreement designed to permit large-scale collective partnering with industry. "These Umbrella CRADAs represent an attractive new way for CCR to collaborate with industry," explained Hale. Instead of a single laboratory negotiating the specific use of a single compound, the CCR Director's Office obtains access to an entire pipeline of compounds for more broadly stated purposes. Under the Umbrella CRADA, individual projects are submitted by CCR investigators in the form of research proposals that are approved by the company. With the legalities attended to, the focus returns to the science.

Advancing Clinical Science

Linehan has devoted 27 years to studying the genetic basis of urologic cancers (see "A War on Kidney Cancer" in Vol. 1, No. 1 of *CCR connections*). He and his colleagues work with families of patients with rare mutations that lead to diseases like von Hippel-Lindau (VHL) and Birt-Hogg-Dube (BHD) syndromes. While treating these rare cases, the Linehan team also uses the knowledge it gains from studying these unique patient populations with known genetic predispositions to kidney cancer to develop better treatments for both familial and sporadic forms of the diseases. "We never promised the families that we would find the genetic basis for their disease," said Linehan. "So you can imagine the

CRADA History

Congress established CRADAs—Cooperative Research and Development Agreements—in 1986 as part of the Federal Technology Transfer Act to allow federal government laboratories to work with industry. However, it took several years to work out the details of implementation, and it has had varied degrees of success across government agencies. With approximately 100 active CRADAs at any one time, CCR accounts for approximately one-half of all CRADAs operating throughout the NIH.

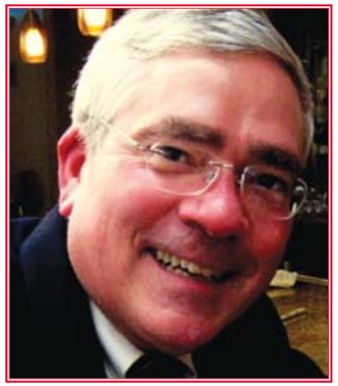
"The CRADA is the only mechanism by which the federal government can promise intellectual property rights to technology," explained Eric Hale, J.D., M.B.A. CRADAs allow companies the first option to license technology and rights that government researchers develop. CRADAs also allow the government to protect from disclosure proprietary information brought into the agreement and protect information emerging from the CRADA for an agreed-upon period. In return, companies can offer research funds and, more significantly, access to proprietary compounds and technologies.

thrill it was both for our research team and for the patients when we succeeded."

Linehan and his colleagues might have declared victory at that stage—how many researchers identify even one cancer-causing mutation in their lifetime? Instead, they turned their focus to the ultimate victory, searching for a curative treatment for kidney cancer.

They have now discovered four kidney cancer genes and developed unique cell lines from their patients that allow them to understand much more about the molecular pathways involved. Yet they still are not able to target many of the proteins in these pathways to develop treatment strategies. "We have found several good candidate molecules by working with the NCI Drug Screening Program in Frederick, which we are encouraged about," explained Linehan. "However, many other promising agents are owned by several different pharmaceutical companies. Having to negotiate individually with companies has really slowed us down."

Across the NIH campus, Mock has been working on elements of the same molecular pathways that Linehan finds affected in kidney cancers, but she approaches them from a different starting point. Mock's group has been studying the genetic predisposition of some mouse strains to the development of plasma cell tumors. "We have uncovered at least four genes that are involved in determining genetic susceptibilities," reported Mock. She and her postdoctoral fellow, Jyoti Patel, Ph.D., have only been working for about three years on pharmacological approaches to studying the pathways they have uncovered, but they immediately ran into difficulties obtaining the drugs that they needed. "In some cases, studying new agents in preclinical settings can be more difficult than doing so in actual clinical trials."



(Photo: Courtesy of G. Curt)

Gregory Curt, M.D.

Both Mock and Linehan are enthusiastic about the Umbrella CRADA with AstraZeneca, which they emphasize has opened the lines of sharing and communication. The investigators meet with company scientists on a regular basis to share ideas and discuss projects. As Beverly Mock observed, "You're not only getting the drug, you're getting interaction with the company that has much preliminary data already gathered for the agent of interest…it's a great way for investigators to network and share protocols and ideas." As Linehan noted, "We have a lot of ideas and the companies also have a lot of great ideas and approaches, and when you get them together, it's magic."

Benefiting Drug Development

The Umbrella CRADA between CCR and AstraZeneca involves what Curt calls "one of our most exciting drugs" whose mechanism of action involves inhibition of mTOR, an important target in cancer research. The agreement covers a suite of drugs against this target and applies to any CCR laboratory or branch. As Curt put it, "We have not just one drug, but drugs and backup drugs. They have not just one branch but many branches. So why should you do a CRADA with the Urologic Oncology Branch only to find out that there's an interest in the Medical Oncology Branch for non-small cell lung cancer (NSCLC) and in the Radiology Oncology Branch for radiation sensitization?" Likewise, if one drug is dropped from development and replaced by another because of bioavailability or toxicity issues, the collaboration does not grind to a halt until a new CRADA can be established.

Like the investigators at CCR, the scientists at AstraZeneca appreciate the extent of the collaboration engendered by the Umbrella CRADA. "The science is shared—particularly, the preclinical science. Neither NCI nor AstraZeneca wants to waste resources by recapitulating the same thing within the company and institution."

Of course, great care must be taken any time a company releases a proprietary compound in drug development to an external organization for research. "We have an obligation as an entity developing a drug to be certain that the work being done with it makes sense," explained Curt. The Umbrella CRADA gives freedom to operate, but individual proposals are still subject to rigorous scientific review before AstraZeneca will agree to move forward. "There is always risk when you give up control of an experimental agent. So you mitigate that risk by working with people that you trust."

Curt sites numerous examples of standard CRADA agreements with NCI that have proven successful. "NCI has found important signals of activity in our drugs that will benefit patients that would have gone undiscovered." For instance, NCI scientists recently discovered that the off-target activity of AstraZeneca's drug Zactima™ is effective in treating medullary thyroid cancer in children. "Isn't it fantastic that one person's off-target effect is a potentially effective treatment for children with an aggressive although rare cancer?"

To learn more about CRADA and CCR's Office of Policy and Intellectual Property, please visit https://ccrod.cancer.gov/confluence/display/OPIPPub/Home.

The Umbrella CRADA:

A Genuine Win-Win Partnership Between NCI and Industry

- Eliminates duplicative research efforts
- Eliminates delays resulting from redundant legal review and approval
- Provides a forum for the exchange of ideas
- Allows for large-scale collaborations
- Allows for the development of multiple agents simultaneously
- Expedites the drug development processes

Nitric Oxide:

Just say NO to Cancer and Much More

Nitric oxide (NO)—a simple molecule consisting of one nitrogen atom and one oxygen atom—is everywhere. Blood vessels use it to relax, neurons use it to communicate, and innate immune cells use it to kill dangerous invaders. NO researchers won the Nobel Prize in 1998, barely a decade after its identification as the biological activity known as endothelial-derived relaxing factor (EDRF). As the initially controversial evidence began to accumulate that NO is a key player in several biological processes, Larry Keefer, Ph.D., Chief of CCR's Laboratory of Comparative Carcinogenesis, was ready with the tools to manipulate it for biomedical research. An organic chemist working on NO-related chemistry as a means to understand and ultimately prevent the carcinogenic effects of the nitrosamines found in a variety of foodstuffs, environmental sources, and manufacturing processes, Keefer was poised to jump into the NO fray with the first reliable chemical donor with which to study the effects of authentic NO in culture and in vivo. Since then, he has initiated several collaborations to develop and study agents that can selectively target NO's power to destroy cancerous cells. His goal is to see one of these agents enter the clinic.

A volatile gas on its own, nitric oxide (NO) is produced where it is needed in the body, and it cannot be directly administered to most biological tissues. Instead, it must be released from other compounds, as in the case of nitroglycerin, which was used to treat heart pain a hundred years before the mechanism producing dilation of blood vessels was shown to be NO.

"My group is [almost entirely] chemists," said Keefer. "We know how to make compounds, make them pure, and design them with specific structural features. That's been our forte. We try to interest collaborators who know how to do the rest of it." One of his most fruitful collaborations has been with University of Utah oncologist Paul Shami, M.D., to study the use of NO to fight cancer.

(Photo: R. Baer)

Larry Keefer, Ph.D.

NO to Leukemia

Shami demonstrated several years ago that acute myeloid leukemia cells are particularly sensitive to NO toxicity at concentrations of a NO-releasing drug substantially lower than those that safely maintain normal healthy endothelial or liver cells.

"NO had long been known as a toxic air pollutant, cigarette smoke constituent, and precursor of carcinogenic nitrosamines. But as its numerous bioeffector roles attest, NO turns out to be essential for proper health just about everywhere in your body," explained Keefer. "So, evolution has provided our cells with ways to deal with its toxic potential."

FEATURE

Shami's leukemia cells had apparently lost some of that ability. This led him to the hypothesis that administering a NO-releasing drug into the general circulation might preferentially eliminate the NO-sensitive leukemia cells without collateral harm to normal tissues. This proved to be the case in a mouse xenograft model of leukemia—a research model in which human cancer cells are grafted under the skin of mice with impaired immune systems to prevent rejection of the foreign graft. The lead compound Shami identified, JS-K, cut the growth rate of the mice's tumors in half without any apparent toxic effects; it also induced more necrotic cell death, relative to controls, in the tumor mass that remained.

Having learned of Shami's success with the *in vivo* leukemia model, Tanyel Kiziltepe, Ph.D., and Kenneth Anderson, M.D., at Harvard Medical School, demonstrated that JS-K also inhibits proliferation of human myeloma cells *in vitro* as well as in xenograft models. Because of JS-K's cell specificity, the doses required to see an effect in mice did not, as expected, cause major changes in vascular tension. The researchers have also studied the mechanisms through which JS-K damages cancer cells and have found evidence for NO-induced DNA damage leading to apoptosis. "I can't put together the whole story on the mechanism yet," noted Keefer. "You look at the structure and chemistry, and there are clearly other pathways by which the compound can be active." Nonetheless, the preclinical evidence is mounting for JS-K's potential as a novel anticancer agent. In a paper published earlier this year in *Leukemia Research*, the team demonstrated that JS-K has a synergistic effect with the antileukemia drug cytarabine in inhibiting proliferation of leukemia cell lines. Shami, in the meantime, has founded a biotechnology company with a confidently optimistic name—JSK Therapeutics.

The efficacy of JS-K appears to extend beyond leukemia and multiple myeloma cells. Similar cytostatic effects have been observed in rodent liver and prostate cancer models. Keefer is also collaborating with Lucy Anderson, Ph.D., Head of the Cellular Pathogenesis Section at CCR, to study JS-K in the multiple human lung cancer cell lines that she and her colleagues have characterized for NO research. Working across the two laboratories, Research Associate Anna Maciag, Ph.D., has unpublished data demonstrating that JS-K is not only effective against lung cancer cells but that it also appears to have an even greater potency in lung cancer cells that have high levels of reactive oxygen species (ROS). "Now we're talking about personalized medicine," commented Keefer. "If a tumor contains high levels of ROS, perhaps it will be an ideal candidate for our drug."

> The preclinical evidence is mounting for JS-K's potential as a novel anticancer agent.

NO Chemistry, Please

JS-K is actually O^2-(2, 4-dinitrophenyl) 1-[(4-ethoxycarbonyl) piperazin-1-yl] diazen-1-ium-1, 2-diolate, a chemical name that looks much more complicated than the guiding principle behind its development. Before stepping into the world of NO biology, Keefer and his team had been working on the related chemistry of a widely distributed class of carcinogens called nitrosamines. As interest in the biological effects of NO grew in the late 1980s, Keefer realized that their knowledge of this chemistry might put them in a unique position to contribute to the field. He knew that compounds of a particular structure—XN(O-)N=O where X is any of a variety of molecular groups—could release NO in a controlled fashion and thought that these compounds could be developed for biological applications.

"So, I took a leap," explained Keefer. He had only recently been through the NIH site visit evaluation process, which takes place every four years, and thus he felt that he could afford to take a chance on this new biology and still turn back if it did not seem fruitful in one or two years.

"The postdoc who started off this work, Tambra Dunams, was very productive," remembered Keefer with respect. "Within the next two years, the NO team we organized including Joseph Hrabie, Chris Maragos, Joseph Saavedra, and David Wink had our first patents, a *Science* paper, and a *Journal of Medicinal Chemistry* paper that is among the most cited in that journal." They tested a series of NO-releasing compounds—diazeniumdiolates or NONOates—and demonstrated that the strength and duration of vasodilation generated by these compounds could be reliably predicted through measurements of their chemical decomposition rates. They then demonstrated a mechanism whereby NO could induce DNA damage by combining with oxygen to disrupt amine groups on DNA directly. "We laid the ground floor for a lot of interesting stuff and are credited with setting worldwide standards for producing reliable fluxes of NO in culture and *in vivo*."

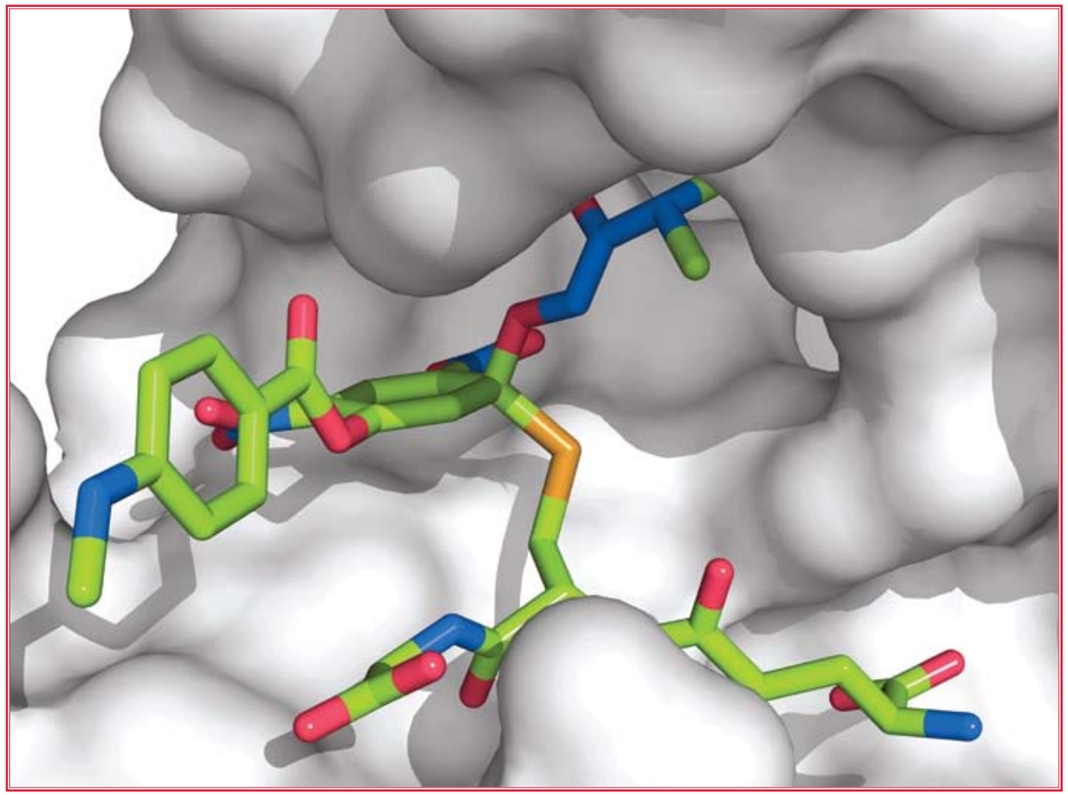
JS-K is just one example of the evolution of that research, and the team is continuing to work on optimizing its composition, chemistry, and delivery. Shami recently presented a study at the American Association of Cancer Research annual meeting describing how JS-K is packaged in lipid nanoparticle micelles to improve its persistence in the bloodstream. Structure-based drug design efforts conducted by glutathione S-transferase (GST) expert Xinhua Ji, Ph.D., of CCR's Biomolecular Structure Section, Macromolecular Crystallography Laboratory, suggested molecular modifications that he predicted would convert JS-K into a particularly effective substrate for the pi isoform of GST, a protein that is overexpressed in a great many tumor cells. Saavedra incorporated these features into a second-generation compound, PABA/NO. Kenneth Tew, Ph.D., now of the University of South Carolina, and his colleagues studied the mechanisms of PABA/NO's cytotoxicity, confirming the involvement of GST and showing that PABA/NO could slow the growth of human ovarian cancer xenografts in mice with a potency rivaling that of the widely used anti-cancer agent cisplatin.

NO, Not Just Cancer

Keefer's early insight that NONOate chemistry might be useful for biomedical research and the conviction that led him to devote two precious years of his four-year NIH review cycle to a project tangential to his prior work have led him from the world of cancer prevention to cancer therapeutics. However, Keefer is quick to point out that since well-regulated fluxes of NO are key to virtually every biological system, the potential for these drugs goes well beyond cancer. "I'm convinced there are lives to be saved and fortunes to be made based on our technology."

NO can be considered a toxic weapon or a cellular defense. Keefer's laboratory is currently studying NONOates that, in addition to directly attacking cancers, could supplement the NO already produced by macrophages to fight disease. They have also designed compounds that will release NO only after they have been activated by cytochrome P450, an enzyme found predominantly in the liver. With it, they hope to increase vascular perfusion during liver failure and protect the organ from ischemic damage. They have also studied the use of these NO donors linked to NSAIDs (nonsteroidal anti-inflammatory drugs) as a means of protecting against the gastric ulcers associated with use of NSAIDs alone. The same chemistry can even form the basis of NO-releasing polymers and materials for use in vascular surgery. The applications are, in short, as widespread as NO itself.

To learn more about Dr. Keefer's research, please visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?profileid=5731.



(*Image: Xinhua Ji, NCI*)

PABA/NO is a NO-donor compound designed to act in tumors overexpressing the enzyme glutathione-S-transferase (GST) pi. GST-pi catalyzes the reaction of PABA/NO with GSH (glutathion) to produce the reaction intermediate shown. The GST active site is illustrated as a molecular surface and the ligand as a stick model in an atomic color scheme *(carbon in green, nitrogen in blue, oxygen in red, and sulfur in orange).*

Inflammation:
Where Immune Cells and Blood Vessels Collide

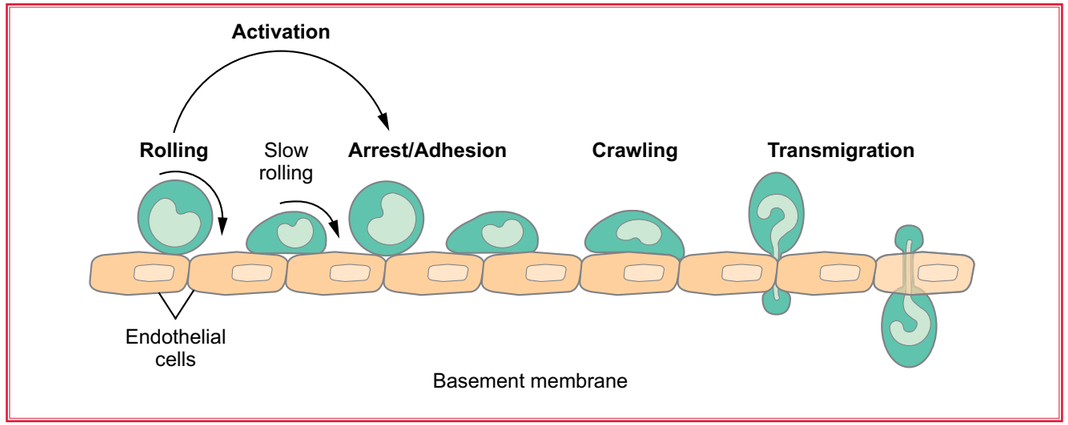
In an average person, there are approximately 20,000,000,000 neutrophils patrolling the bloodstream on the lookout for trouble. These foot soldiers of the immune system represent a first line of defense against foreign assault from a variety of pathogens. As they circulate through the body, neutrophils might find themselves slowing down in thickening blood and brushing up against blood vessel walls where the tissue has been inflamed by infection or disease. This first casual interaction with the endothelial cells that line blood vessels begins a complex cascade of intercellular molecular interactions that ultimately propels the neutrophils out of the vasculature and into the damaged tissue where they can wreak havoc on the enemy before committing hara-kiri. Neutrophils are just one of several types of white blood cells—or leukocytes—that must interact with vascular endothelial cells to execute their immune functions. Triantafyllos Chavakis, M.D., Ph.D., Head of the Inflammation Biology Section in CCR's Experimental Immunology Branch, is studying the molecular basis of leukocyte-endothelial interactions with the goal of finding ways to suppress the damaging inflammatory response that characterizes inflammatory and autoimmune diseases.

Tissue becomes inflamed—whether because of a bug bite or arthritis—when chemical signals elicit the blood vessel changes necessary to recruit leukocytes to the site of injury. Neutrophils, for example, have specific receptors that detect and respond to certain bacterial proteins; other leukocytes respond to chemical signals known as chemokines that are released by cells in the area as a kind of general alarm. Although inflammation does not literally set tissue on fire, like its namesake, it must be deployed carefully lest it cause more harm than good. Autoimmune diseases such as multiple sclerosis and rheumatoid arthritis are caused by a misguided attack from the immune system on host tissue, which includes inflammation as a key destructive force. Neurodegenerative diseases like Alzheimer's also appear to have a strong inflammatory component. In such cases, although inflammation itself may not be the root cause of the disorder, stopping it can be a strong defense. Indeed, leukocyte inhibitors are already used to treat psoriasis and multiple sclerosis. "Whether you study inflammatory disease or autoimmunity," noted Chavakis, "an essential component is the leukocyte—if you block this, you could target disease."

The Leukocyte-Endothelial Interaction Cascade

Endothelial cells maintain a physical barrier that allows for the efficient circulation of blood as well as metabolic exchange with surrounding tissue. Leukocytes are free to travel these passageways, but leaving them requires an active multistep molecular signaling cascade that engages both the leukocyte and the endothelial cells.

The process by which a leukocyte leaves the bloodstream to enter a tissue is generally thought of as occurring in three



(Image: J. Kelly)

The leukocyte-endothelial interaction cascade. Leukocytes leave the blood vessels through a multistep process beginning with rolling and ending in transmigration through the endothelial cells.

> "Whether you study inflammatory disease or autoimmunity," noted Chavakis, "an essential component is the leukocyte."

phases: rolling or tethering, activation, and then adhesion and transmigration, which have both mechanical and molecular signaling aspects. In the first phase, a leukocyte will literally bump up against the endothelial cells forming the blood vessel. This interaction results in a weak binding between molecules known as selectins on the surface of the leukocyte and their counterparts on the endothelial cells. Once loosely tethered through selectin-binding, leukocytes are exposed to chemokines at the endothelium that are produced during inflammation. Chemokines then activate the leukocytes to bind more tightly to the endothelial surface through a different set of receptors—integrins. Integrin-binding enables the leukocytes to crawl along the blood vessel seeking a point of exit. More often than not, this exit occurs at a junction between endothelial cells where a different set of molecular interactions guides the leukocyte through the normally sealed barrier.

Cascade Inhibitors Identified

As a doctoral and postdoctoral student and later as a practicing clinician, Chavakis conducted research in the laboratory of Klaus Preissner, Ph.D., in Germany. His interest in the biology of leukocyte-endothelial interactions was stimulated, in part, by the patients he saw, including many with diabetic complications such as wounds that refused to heal and that became chronically infected by bacteria. However, his research was inspired not as a direct challenge to infection but from a desire to copy bacterial strategies for subverting inflammation. "We weren't really studying how innate immunity copes with bacteria," remembered Chavakis, "but how bacteria can avoid the innate immune response."

The bacteria *Staphylococcus aureus* have adapted themselves to successful human infection through a range of strategies including the production of factors that interact with host proteins to assist in bacterial colonization and propagation. In 2002, Chavakis and his colleagues identified Eap (extracellular adherence factor)—a protein secreted by bacteria to block the innate immune response by inhibiting recruitment of neutrophils. They found that Eap interacted with ICAM-1 to prevent the adhesion of leukocytes required for their translocation to the site of infection. They went on to demonstrate that Eap could dampen the autoimmune response in a mouse model of multiple sclerosis. In the meantime, *Staphylococcus* has been found to produce inhibitors that target each step in the leukocyte-endothelial interaction cascade.

Upon moving to NCI, Chavakis shifted his focus away from bacterial effectors to study intrinsic mechanisms of innate immunity. However, he was surprised to discover that leukocyte inhibitors were not so easily dismissed. In a paper published in *Science* in 2008, Chavakis and his colleagues demonstrated that a previously known glycoprotein—Del-1 (Developmental endothelial locus-1), which had been implicated in blood vessel remodeling—had an important novel role in leukocyte-endothelial adhesion.

"If you look at the leukocyte adhesion cascade," explained Chavakis, "you will find maybe 20–30 receptors generally working to promote adhesion. Very few adhesion proteins do the opposite." Although Del-1 had all the hallmarks of a protein that bound to the adhesion machinery of leukocytes, it appeared to block adhesion instead of promoting it. They found that mice lacking the *Del-1* gene demonstrated increased leukocyte adhesion and accumulation of neutrophils when challenged in a model of lung inflammation. Furthermore, the researchers were able to show that Del-1 was produced at high levels in parts of

the body that tightly restrict access by the immune system, like the brain and the eyes.

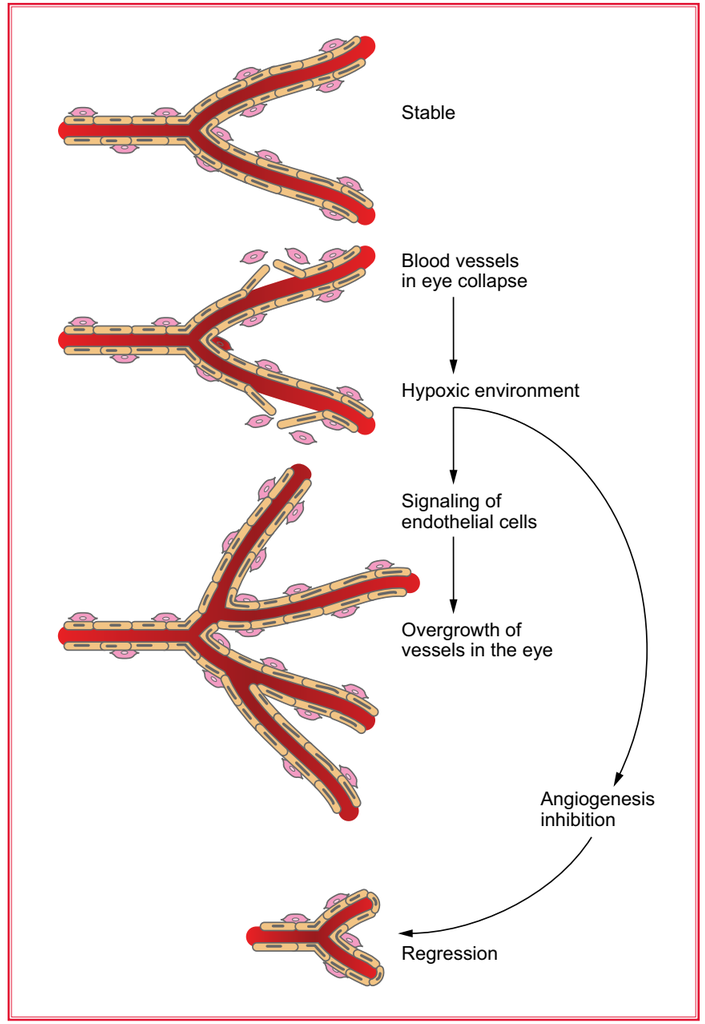
Currently, they are challenging the *Del-1* knockout mice in a model of multiple sclerosis. "But it would be even more interesting if we could generate bioavailable forms of the protein and see if it could really inhibit inflammatory disease," noted Chavakis. The team is currently working with NCI's Protein Purification Laboratory to render the Del-1 that they are producing in cell cultures fit to test in animal models.

A Small Step to Angiogenesis

From studying the vascular changes that occur during inflammation, it is not a large experimental leap to study the formation of new blood vessels in developed tissue since both involve initial changes in vascular permeability and many of the same molecular factors. One of the key initiators of vascular growth is a lack of oxygen.

Chavakis's lab works with a particular model of retinopathy in mice that has strong parallels with a disorder that occurs in babies born prematurely. Because their lungs are not sufficiently developed, these babies often require high oxygen environments, which unfortunately damage the retinal vasculature. In mice, the retinal vasculature develops normally in the first 15 days after birth, but a high oxygen environment in the second week destroys the retinal vessels. Once you return the mice to a normal oxygen environment, the resulting hypoxia causes the disorganized pathological vessel growth that is characteristic of many other forms of retinopathies as well as cancers. This model is both physiologically faithful to human disease and nicely accessible to study; in collaboration with researchers at the National Eye Institute, Chavakis hopes to be able to inject compounds directly into the eye to observe their effects on neovascularization.

Chavakis estimates that his efforts are currently split two-thirds/one-third between leukocyte-endothelial interactions and angiogenesis, but his interest in both is stimulated by the clear overlap between the two areas of study. Recently, a molecule that Chavakis helped to identify while he was still in Germany for its role in leukocyte transmigration has reappeared in the laboratory under the more general guise of altering vascular permeability.



(*Image: J. Kelly*)

Model of hypoxia-induced retinopathy. The collapse of blood vessels in the eye results in a lack of oxygen delivery to the tissue. This hypoxic environment generates signals that cause abnormal blood vessel proliferation, which can be suppressed by angiogenesis inhibitors.

Junctional adhesion molecule-C or JAM-C, as it is now known, first came to Chavakis's attention through a colleague, Sentot Santoso, Ph.D., who was immunizing mice with human platelets in order to study the molecules responsible for an autoimmune disorder known as immune thrombocytopenia. "He had tons of nice antibodies and some of them reacted with new unknown targets," recalled Chavakis. The two researchers began talking and eventually working together when it became apparent to Dr. Santoso that one of the targets had a sequence very similar to the only known junctional adhesion molecule at the time. "So we immediately picked it up to see if it was in endothelial cells and, in fact, the first thing that we published was that it binds to integrins on leukocytes." They and others have gone on to demonstrate that JAM-C localizes to junctions between endothelial cells and regulates the ability of leukocytes to pass between them.

In a paper published in *The Journal of Experimental Medicine* in 2006, Chavakis's group went on to demonstrate that JAM-C has a somewhat counterintuitive role in inhibiting adhesion between endothelial cells. "We found that JAM-C regulated junctions in the opposite manner: by removing the protein, the junctions became better." Disrupting JAM-C caused a decrease in permeability and a dampening of the normal increase in permeability caused by histamine or vascular endothelial growth factor (VEGF). It also decreased the extent of aberrant new blood vessels in their mouse model of retinopathy. Chavakis and his colleagues are currently working on a conditional knockout of the gene in mice to further study its functions *in vivo*.

Homeostasis: What Is Normal?

Chavakis is intrigued by the fact that, depending on the tissue examined, the blood vessel endothelium can have vastly different functions. In the lung, it must regulate oxygen transfer and resist the temptation to mount an inflammatory response to every foreign agent that is inhaled. In the brain, it must protect the neural tissue from a variety of molecules that are free to pass through virtually any other tissue in the body. The blood-brain barrier also resists invasion by cells of the immune system, whereas, in the liver, the blood vessels are open and fenestrated to allow a much greater exchange of molecules. "Obviously, the endothelium in each case is adapted to the function of the tissue," commented Chavakis. "But why and how?" These tissue differences are only starting to be considered experimentally, yet it is likely that the leukocyte-endothelial interaction cascade itself is different depending on the tissue type.

> Depending on the tissue examined, the blood vessel endothelium can have vastly different functions.

"There is a perception that you easily adopt if you read big reviews in this field that leukocytes themselves only promote vascular growth, but if you start doing experiments, you sometimes end up with different results," noted Chavakis. "And then, there is a smaller piece of literature, which is easy to ignore, showing that certain leukocytes probably do the opposite." Chavakis is more and more convinced that these seemingly contrary findings will prove important to understanding vascular integrity.

More difficult to study than the processes stimulated by inflammation, Chavakis wants to find a way to study the normal homeostatic mechanisms that maintain blood vessels. In the absence of infection or disease, blood vessels are generally quiet. Unlike many organ systems that have a high turnover of cells in their tissue, healthy blood vessels maintain their integrity without much fanfare, resting shoulder to shoulder to provide a safe passageway for the blood. However, this in itself is a mystery—how do blood vessels maintain their integrity in the face of the physical stresses they encounter? Every day, all day, blood cells stream along the vessel lining, yet they do not wear it down. Chavakis is convinced that some of the molecules he studies could be actively involved in maintaining this perceived quiescence.

(*Photo: R. Barr*)

From left to right: Triantafyllos Chavakis, M.D., Ph.D.; Harald Langer, Ph.D.; Eun Young Choi, Ph.D.; and Valeria Orlova, Ph.D. Missing from the Chavakis team photo: Sunil Kaul, Ph.D.

> "Immunologists don't consider vascular biology, and vascular biologists don't study immune systems. A few of us are happy to be somewhere in the middle."

The challenge will be to study it. "In contrast to conditions like tumor angiogenesis or retinopathy, how do you create situations to manipulate vascular maintenance in which you don't address proliferation? We are still trying to find out how to do it." In the meantime, Chavakis will continue to mine the rich intersection between immune and vascular function. "Immunologists don't consider vascular biology, and vascular biologists don't study immune systems. A few of us are happy to be somewhere in the middle."

To read more about Dr. Chavakis's research, please visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?profileid=10637.

Breast Cancer Genes:

When the Sequence Is Not Enough

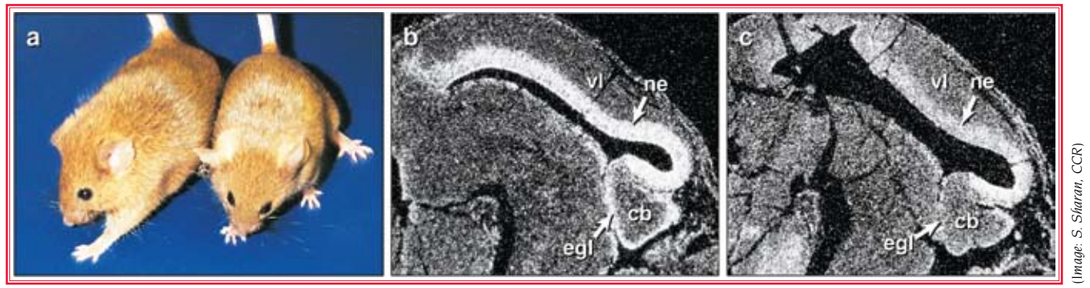
Few cancer genes are more notorious than the genes that cause familial breast cancer—BRCA1 and BRCA2. The New York Times described the cloning of BRCA1 in 1994 as "a genetic trophy so ferociously coveted and loudly heralded that it had taken on a near-mythic aura," but cautioned that since the gene was unexpectedly large, it might take at least a year before a diagnostic test could be developed from it. Fifteen years later, there are indeed genetic tests to evaluate the risk of breast and ovarian cancer in women who possess one of several known mutations. There are, however, even more variants for which the risks are not yet understood. Shyam Sharan, Ph.D., Senior Investigator and Head of the Genetics of Cancer Susceptibility Section in CCR's Mouse Genetics Cancer Program, understands the difficulties of studying these genes better than many. As a Postdoctoral Fellow, he got caught in the race to understand the BRCA genes by cloning their mouse homologues. That initial sprint turned into a marathon, and although it is far from over, the recently tenured Sharan appears exhilarated by the milestones he has recently passed.

In the four-year period after Mary Claire King's groundbreaking identification of a region of human chromosome 17 linked to familial breast cancer, *BRCA1* and *BRCA2* were cloned in humans and mice, and their function was linked to DNA repair. This pace was a source of optimism for the field, intense competitive pressure for the scientists involved, and occasional humor. "I went to a Keystone meeting on breast cancer and gave a talk," remembered Sharan. "After me, Thomas Ludwig also gave a talk about a *Brca2* knockout mouse, and as a joke, I got an award for winning the *Brca2* race by 15 minutes." However, identifying the genes turned out to be only a first step in both understanding their role in tumorigenesis and predicting which mutations would be oncogenic.

(*Photo: R. Bazer*)

Shyam Sharan, Ph.D.

In particular, two puzzles from that time have continued to drive Sharan's research. The first led from the observation that the known mutations did not seem to cluster into any "hot spots" but were distributed throughout the gene, suggesting that all regions of the protein were equally important for tumor suppression. With 1,863 amino acids and 3,148 amino acids respectively, BRCA1 and BRCA2 are huge proteins (for comparison, hemoglobin, which carries oxygen in the blood, has 574 amino acids). Sharan and others had identified associations of these proteins with DNA repair, but that was far from a complete functional explanation of these complex proteins. What did the rest of these proteins do, and how did far-flung mutations contribute to tumorigenesis? The second puzzle stemmed from the seeming paradox that eliminating either protein from mammary cells resulted in cancerous proliferation, whereas disrupting them in embryonic mouse cells resulted in a failure to proliferate and develop. How could a gene involved in something as basic to the cell as DNA integrity cause opposite effects in different cell types?



(*Image: S. Sharan, CCR*)

Human *BRCA1* is fully functional in mice, and its expression mirrors the mouse *Brca1* gene. In panel a, the *Brca1* mutant mouse *(right)* rescued by the human *BRCA1* BAC transgene appears indistinguishable from its wild type littermate *(left).* Panels b and c show an expression analysis of the human *BRCA1* transgene *(panel b)* and endogenous *Brca1 (panel c)* in the brain of a 13.5-day mouse embryo. High level of expression was observed in the neuroepithelium (ne) of the ventricular layer (vl) and the external germinal layer (egl) of the cerebellum (cb), as shown by the arrows.

> What did the rest of these proteins do, and how did far-flung mutations contribute to tumorigenesis?

Sense through Missense

When Sharan came to NCI, he wanted to use mouse genetics to study the functions of the BRCA genes. He knew that most of the identified mutations in BRCA genes came from tumor samples, and it was therefore not surprising that they resulted in tumorigenesis, but Sharan wanted to be able to mutate regions of interest in these genes systematically to study their effects. However, there was a small problem—the mouse and human *BRCA1* genes are only about 60 percent homologous, which means that, in mice, the human gene of interest is already mutated by 40 percent. Nevertheless, Sharan decided to introduce the human *BRCA1* gene into mice. And not just the gene, but the entire 200,000 base-pair length of human DNA that comprised all of the regulatory elements as well as the gene itself.

"It was kind of risky," commented Sharan, noting that for the experiment to succeed, the mouse cells would need to contain the necessary cellular machinery to properly regulate the human elements, which was by no means clear. Indeed, a paper that came out just as they were making the first mice examined the regulatory elements in a 2,000 base-pair region of the mouse and human genes without finding any obvious conservation between the species. "But we wanted to express the gene at physiological levels and not hook it to a promoter that would overexpress it...and it actually paid off." The human DNA was able to completely mimic—or rescue—the missing mouse *Brca1*. Most exciting, the expression pattern of the human gene in these mice was exactly the same as the normal mouse gene, which is expressed ubiquitously in early development and then downregulated in cells that begin to differentiate.

However, the goal was to study mutations introduced into the *BRCA1* gene. With mouse model in hand, the investigators' next hurdle to overcome was to be able to make targeted point mutations in a large genetic sequence before creating the mouse. Here, Sharan had the help of his colleagues down the hall—Neal Copeland, Ph.D., Nancy Jenkins, Ph.D., and Don Court, Ph.D.—who had recently developed just the recombineering technology he needed to adapt into his own system (see "Science in Singapore: Aiming High for Biomedical Research," page 26).

"As we started to make mutations, we quickly learned two lessons," explained Sharan. The first was that mutations that were supposed to be deleterious based on their location in highly conserved (and hence arguably important from an evolutionary standpoint) regions of the gene often had no effect on the mice. Even biochemical data showing disrupted protein-protein interactions of the mutated *BRCA1* could not predict an abnormal phenotype in the mice. The second thing they learned was that several of the deleterious mutations were a result of altered splicing of the gene, effectively knocking it out completely. So, it was impossible to simply look at the amino acid sequence and predict the impact of a single mutation. Every mutation had to be studied individually.

"You can imagine how this could impact my career and my postdoc's career. Making mice with no phenotypes is not exactly exciting." The researchers tried everything they could think of to show the effects of their mutations—they aged the mice, made fibroblast cultures from them, and studied them biochemically. And yet, they still found that many of their mutations had no obvious phenotype. They needed to find a better way to screen mutations and to know that what they were looking at were important clues to *BRCA1* function and not just a difference between mice and men.

Embryonic Stem Cells Tell All

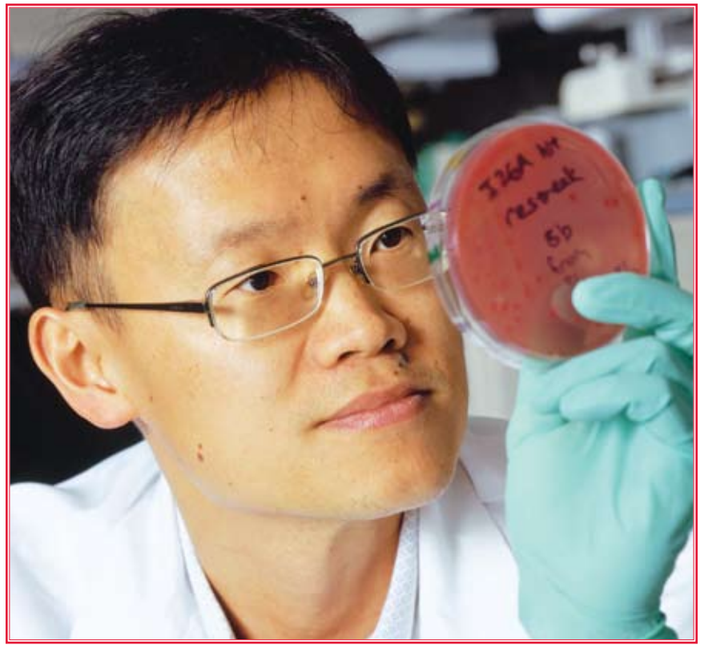
To generate *Brca1* or *Brca2* knockout mice, the first step was to make mouse embryonic stem (ES) cells in which one copy of the gene is disrupted by gene targeting technology. While they were waiting for the

mice, Sharan, at that time a Postdoctoral Fellow in the laboratory of Allan Bradley, Ph.D., at the Baylor College of Medicine in Houston, thought a relatively easy and straightforward next step would be to make mouse ES cells with both copies of the gene missing in order to study the resulting defects. The problem was that he just could not get ES cells that were missing both copies of the gene to survive.

"I wasn't doing anything with these cells," recalled Sharan, "when I one day realized that they could be a powerful system." He recognized that if he made the remaining mouse copy conditional—so that it could be deleted at will—and added in the human gene sequence, the ES cells would only have one human BRCA gene remaining once he deleted the second mouse copy. How the cells behaved with only the human *BRCA* gene with or without mutations could tell them a great deal about the individual mutations. If the mutation was neutral, then the cells should survive; if it was deleterious, the cells would die. And with any luck, there would be a range of phenotypes, depending on the specific mutations, that would not affect survival per se but would affect DNA repair or other cellular functions when tested.

Sharan and his Postdoctoral Fellow, Sergey Kuznetsov, Ph.D., first tested founder mutations of *BRCA2*—those highly specific mutations found in families that have remained relatively genetically isolated—that are strongly linked to breast cancer. As expected, the ES cells did not survive. Then they tried mutations or variants that are frequently found in the general population and are, therefore, thought to be neutral. The ES cells appeared normal. Finally, they tried mutations that they thought might be deleterious based on the available literature. As they had found in their mouse models, the majority did not show an effect in their cell-based assay. "We were kind of depressed," Sharan explained candidly. "We thought that the assay might not be sensitive enough."

He then decided to contact Myriad Genetics, the company that first cloned and patented the human *BRCA1* and *BRCA2* genes to develop genetic tests. Myriad Genetics had a database of human mutations from breast tumors



(*Photo: R. Bazer*)

Postdoctoral Fellow Suhwan Chang, Ph.D.

that included data on whether or not the specific mutation was genetically associated with cancer in families and whether other deleterious mutations were found in the gene that might instead be responsible.

"And that was one of the best days," said Sharan, smiling at the memory. "In every single case, our data matched with the family segregation or linkage data. Where we found it to be neutral, the family data showed it was not segregating with the disease or, in some cases, they found additional deleterious mutations."

Kuznetsov and Sharan continued to develop their assay and tested several additional mutations. In every case, their results matched the outcome predicted from human data. "For a long time, we knew that the assay was working—we were convinced—but we only had cases in which the cells would die completely or they would be normal," explained Kuznetsov. To dissect the functions of these genes, they needed to find mutations that were not so severe that they would cause ES cell death but that could be measured as abnormalities in the cells. Eventually, they found some examples of such intermediate mutations, but the most memorable example came while the paper they eventually published in *Nature Medicine* in July 2008 was still under review.

> These same techniques could be applied to many diseases.

One of the mutations they had studied resulted in a single amino acid substitution of tryptophan for arginine, which they found to be deleterious. A reviewer of the paper was aware of a human variant at this same nucleotide that would change the arginine into a glutamine and, according to the literature, be neutral. "In principle, we could have argued that the phenotype would be different [depending on the exact mutation]," said Kuznetsov, but the researchers instead took it as a challenge. Could they assay a specific mutation in the three months allowed by the journal for resubmission? The answer turned out to be more interesting than either expected. The cells survived, as predicted by the reviewer, but they had subtle defects depending on the drug researchers used to challenge the cells. However, when they went back to the paper proposing that this mutation was neutral, the researchers realized that it was actually a borderline case according to the scale used by the authors. Furthermore, they were able to show through structural modeling that although the second mutation did not disrupt the conformation nearly as much as the first mutation they had tried, it did not leave the protein undisturbed, arguing for the possibility of subtle functional defects. The resubmitted paper was accepted without further ado.

Sharan and his Research Fellow, Suhwan Chang, Ph.D., have now developed a similar assay for mutations in *BRCA1*—work that faced its own unique challenges—that is published in the October 2009 issue of *The Journal of Clinical Investigation*. The assays are licensed through NCI and are available as a research tool for clinical scientists interested in characterizing additional human variants of the BRCA genes. Sharan is hopeful that in a world of increasingly available genetic testing, his assays will ultimately help inform the risk of disease. "Our approach is not just limited to *BRCA1* and *2*. As long as there is a phenotype that can be studied at the cellular level, these same techniques could be applied to many diseases."

Variations on a Theme

Although they believe they now have the tools to test any mutation in BRCA genes, Sharan's goal is not to catalog BRCA mutations. "Understanding why the mutations are deleterious—that's what I wanted to do, and it's taken me 10 years to get there."

Sharan is grateful for the support he has received to allow his research to mature. "This took a long time. In most cases, you get more support once things are published and if it's in a good journal...so I feel extremely grateful to Bob Wiltrout [Director of CCR] who gave me more resources before our work was recognized by others."

Sharan is particularly interested in the intermediate phenotypes that they have discovered through their assays—mutations in *BRCA1* and *BRCA2* that show subtle signs of chromosomal instability and other cellular abnormalities. In addition to further testing in ES cells, Sharan and his team are planning to return to making mice in order to analyze their most interesting mutations in a whole animal. Chang is focused on *BRCA1*, whose amino acid sequence reveals multiple functional domains. "It is a really interesting project to study because it's been more than 10 years since *BRCA1* was discovered and people have put a lot of effort into studying it, but we still don't know its function."

Sharan also wants to return to the second half of the puzzles that have driven him from his postdoctoral work to his current position—namely, why BRCA mutations have different effects depending on the cell type. "We now have a very simple system—we have ES cells that are dying. What genes are there that make the cell die instead of survive?" His plan is to use the ES cells with altered BRCA as a screen to find other genes that are involved in BRCA-dependent survival. With no shortage of new avenues to pursue, Sharan is aware of the need to stay focused. "Do it right, and slow but steady will win the race."

To learn more about Dr. Sharan's research, please visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?profileid=5567.

(*Image: S. Sharan, CCR*)

Betty K. Martin, Suhwan Chang, Ph.D., and Kajal Biswas, Ph.D., *(left to right, back row)* and Dr. Shyam Sharan, Ph.D., and Susan Lynn North work together to pursue the functions of the *BRCA1* genes.

Science in Singapore:

Aiming High for Biomedical Research

After 22 years at NCI, Nancy Jenkins, Ph.D., and Neal Copeland, Ph.D., left the Mouse Cancer Genetics Program they built at CCR to start a new adventure halfway around the world in Singapore's Institute of Molecular and Cell Biology (IMCB). In Singapore, the husband-and-wife team saw a unique opportunity to shape a new and exciting research enterprise in a region of the world they had both enjoyed so often as visitors. Although they were recruited to co-direct the Division of Genomics and Genetics, Copeland became the Executive Director of the Institute within a year. The IMCB is now part of a complex known as Biopolis, which includes several institutes that are conceptually similar to divisions of the NIH intramural program. However, the campus also houses the research and development operations of several pharmaceutical companies as well as fosters nascent biotechnology companies—an innovation that Jenkins and Copeland enthusiastically support for the perspective and talent it brings to translational research. The genetic models of cancer they created while at NCI and the insights derived from them have been widely recognized as seminal contributions to the field and were recognized and honored this year by the couple's election to the U.S. National Academy of Sciences.

A New Beginning

Our institute—the IMCB—is the oldest in Biopolis. It was founded in 1987, which doesn't sound that old until you consider that it is exactly half as old as the independent country of Singapore itself. In fact, it was only in the 1980s that Singapore attained the kind of economic prosperity that could support a strong research enterprise; however, the current enthusiasm and support for scientific research and development is remarkable. There is a definite feeling in the air that Asian science is on the rise, and those countries that can afford it—like Singapore—are pinning their economic future to scientific innovation. They are investing heavily, recruiting outstanding people, and giving them great resources.

The Nobel prize-winning developmental biologist Sydney Brenner, Ph.D., was instrumental in advising the government of Singapore to build the IMCB as a means to further basic science research and training. It was patterned originally after the Laboratory of Molecular Biology in Cambridge, U.K., one of Brenner's professional homes, which remains a special place today in no small part because—like the NIH—they have had "hard" funding directly from the government with which to recruit and support the best people.

Funding for the IMCB comes directly from Singapore's Ministry of Trade and Industry. The institute comprises approximately 400 people in 30 labs implementing a range of basic research programs in cell biology, structural genetics, bacteriology, infectious disease, and of course cancer. The IMCB has a strong infrastructure for research in model organisms, including the largest zebra fish facility in South East Asia with approximately 9,000 fish tanks. Organizationally, the institute is divided into four divisions: Genomics and Genetics, Genes and Development, Systems Biology, and Cancer and Developmental Cell Biology, each headed by a deputy director. The divisions are actually a relatively recent invention, implemented by the previous Executive Director, Sir David Lane, who found a monolithic structure increasingly cumbersome as the institute grew in size. The directors are responsible for dispersing funds to investigators who are all on three- to five- year contracts. We make decisions about whether contracts will be renewed and the levels of funding each laboratory will receive in conjunction with an international scientific advisory board that helps us to review the progress and potential of our laboratories.

(Photo: Courtesy of N. Copeland and N. Jenkins)

Nancy Jenkins, Ph.D. *(left)* and Neal Copeland, Ph.D. *(right)* continue their innovative cancer research at the Institute of Molecular and Cell Biology in Singapore.

> Biopolis is a place that is striving to fuse basic science and translation without adhering to traditional fire lines.

A Growing Biopolis

In 2004, the IMCB moved from its original home at the National University of Singapore to the newly constructed Biopolis campus. Still in its first phase of development, Biopolis currently has nine buildings. Next door to our institute is the Genome Institute started by Edison Liu, M.D., who also moved from CCR where he served as Clinical Director. In addition, Biopolis includes recently formed institutes in the areas of bioinformatics, nanotechnology, immunology, and medical biology.

The massive infusion of funding for Biopolis is, in part, tied to a strategy designed to attract pharmaceutical research and development. Singapore is already a major place for pharmaceutical manufacturing, but the goal is to create a rich environment for innovation as well as production. Biopolis provides space for pharmaceutical companies, which includes access to all of the core facilities (for a fee, of course); about half the space is currently occupied by companies including Eli Lilly and Company, Schering-Plough, Novartis, and GlaxoSmithKline. The Singapore government also supports nascent biotechnology companies that spin off research developed in the country.

In short, Biopolis is a place that is striving to fuse basic science and translation without adhering to traditional fire lines separating academic and private research. When it comes, its success in fostering truly collaborative translational research will rest not only on this physical juxtaposition but also on changes that we see occurring in the pharmaceutical industry itself. The old bunker mentality of huge, secretive in-house research facilities seems to be giving way to a much more open and interactive mode of operation in which pharmaceutical companies are increasingly seeking out academic collaborators and partners. Everyone is realizing that the science needed to support successful drug development, from making the best compounds to developing the best assays, is too complex to go it alone.

Our Ongoing Research

For all of the changes we have seen in the last few years, one thing that hasn't changed for us is the direction of and vision for our own laboratory's research program. Right before we left NCI, we developed a new technology that allows us to induce many different cancers in mice. Previously, our focus had been on the hematopoietic cancers that we could produce using retroviruses and insertional mutagenesis, but we always felt limited because we were not able to study the solid cancers that are so much more common in people. Then we developed a technique that uses a transposon called Sleeping Beauty (SB)—a piece of DNA isolated from salmon that can be introduced into mouse genomes and mobilized so that it jumps from where it was originally inserted into another random part of the genome. If it disrupts one of the mouse genes and induces cancer in the process of re-insertion, we can quickly clone the gene responsible because the transposon also serves as a molecular tag. It is an incredibly powerful technology, and we can not only mobilize the transposon in all cells, but we can also selectively mobilize it in specific tissues and thereby model different organ tumors. Through this technology, we have set up many cancer models and also sent these mice to laboratories around the world for collaborations. The technology is still new, and we are working on ways to improve it. Our goal is to identify not only cancer genes but also new drug targets for human cancers—work we hope to do in partnership with the pharmaceutical companies that have come to Singapore.

In fact, there are very few places in the world apart from CCR where we could continue our life's work. And the NIH intramural program is really the only place we could have begun it. We could never have written enough grants to do all of the preliminary work that led to our transposon system. The project involved a sustained commitment of large-scale resources and high-risk science over the course of many years. At one point, we had 9,000 cages of mice in our facility in Frederick, and this scale was absolutely necessary to making the discoveries that we did.

An Evolving Institute

As we think about the future of the IMCB, we want to ensure that, like NCI, it is a place where investigators can think big and take risks. Singapore is continuing to invest heavily in science, and Biopolis is growing every day, but billions of dollars have also gone into new research programs for the universities. How do we distinguish our unique strengths? One way—although not the only way—is to focus on large interdisciplinary projects that are difficult to do in the university research environments where research funding is largely structured around individual investigator-initiated programs. Another method is to aim high at the kind of translational research that academia typically encounters difficulties in trying to achieve. In any case, the goals of the IMCB as it matures will reflect the development of Biopolis and of science across Singapore and South East Asia. It's an amazing, dynamic place to be.

Cut to the Cure

As a staff clinician in the Surgery Branch of CCR, Marybeth Hughes, M.D., is involved in a number of different collaborative projects aimed at improving the care of cancer patients. In some cases, she is looking for ways to apply an innovative surgical technique to treat a particular form of cancer. In others, a well-established procedure is a means to both excise and study a tumor for molecular and genetic clues to its origin and, hopefully, its cure. When she is not in the operating theater, Hughes may be found conferring with her bedside colleagues over symptoms of an unidentified endocrine tumor, working with her benchside colleagues to develop effective immunotherapies, leading a multicenter clinical trial for the treatment of liver metastases, or teaching medical students at the Uniformed Services University of the Health Sciences. The common thread running through the diversity of her professional activities is a desire to push the limits of understanding and treatment of cancer in its many guises. Hughes attributes her decision to specialize in surgical oncology to a combination of personal experience with unusual forms of cancer in her family and positive mentoring at critical junctures in her education.

(Photo: R. Baer)

Marybeth Hughes, M.D.

Tracking Endocrine Tumors

Although we may imagine a molecular-medicine future in which oncologists administer a smart pill to deliver a targeted treatment exactly and only to cancerous cells, right now, surgery is still the only option to cure cancers in many settings. In part, that is because many cancers are so rare that we know surprisingly little about them.

Endocrine tumors are a lesson in just how complex our physiology really is. Abnormal cells producing a variety of hormonal or neural signals—ranging from insulin to serotonin—can be lodged virtually anywhere in the body, and the resulting symptoms such as flushing, diarrhea, or abnormal blood sugar levels can be misdiagnosed for years. Even when biochemical analyses correctly reveal the presence of a tumor, finding it can still be a challenge (see "Where Is the Tumor?" page 32). Intellectually, the diagnosis of these tumors can be fascinating and ultimately satisfying because surgical removal has a high success rate in many cases, particularly when the tumors are caught early.

Because we have some of the world's foremost endocrinologists here at the NIH, we see some very unusual cases, and I often provide surgical consults for their treatment. Karel Pacak, M.D., of the National Institute of Child Health and Human Development, world expert on pheochromocytoma, consults with me about surgical removal of this rare, tumor of the adrenal gland. Constantine Stratakis, M.D., D.Sc., Head of the Program in Developmental Endocrinology and Genetics, studies a rare, inherited form of adrenal disorder known as primary pigmented nodular adrenocortical disease (PPNAD), which causes affected children to go through cycles of high cortisol levels that produce dramatic changes in weight. The only treatment for both of these disorders is surgical removal of the adrenal glands.

As has proven the case for many other types of cancers, we believe that familial patterns of endocrine tumors might teach us not just about these rare cases but about the more prevalent, sporadic forms of endocrine cancers as well. I am also currently working with Steve Wank, M.D., Chief of the Digestive Diseases Branch at the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), to study and treat familial carcinoids, slow-growing tumors that secrete serotonin to cause flushing and digestive complaints but usually only after the disease has advanced to liver metastases. Some patients are fortunate—they may have had a carcinoid in the small bowel that bled, prompting a capsule endoscopy (essentially, a camera in pill form) study that locates the tumor. Many are not properly diagnosed until their disease has metastasized to the liver, and the prognosis is poor. We recently saw a patient whose mother, uncle, and brother had all died of the disease. A capsule endoscopy revealed small tumors in her intestines, which we have removed surgically.

We hope that, when surgically removed early, these carcinoids will not have an opportunity to metastasize to the liver. However, because it is often diagnosed so late, it is difficult to understand the natural history of the disease or how effective treatments may be when administered early. We do not know, for instance, whether the carcinoid is part of a "field defect" involving a whole segment of the bowel or whether it is isolated to the ostensibly abnormal cells. Thus, in addition to simply excising the tumors, we are also analyzing single nucleotide polymorphisms (SNPs) and gene expression patterns in the tumors themselves and the adjacent tissue. Without an identified genetic defect, we are still trying to determine the best way to screen for the disease.

In general, endocrine surgeries require a great deal of patience because the tumors are often embedded in neovasculature that must be painstakingly worked through. The dissection can be tedious. As in every other medical speciality, surgeons must also keep abreast of new technologies that may improve outcomes for our patients. For some types of endocrine tumors, advances in minimally invasive surgical techniques can not only be of cosmetic benefit in reducing scarring, but they can also provide real treatment benefits through better visualization and easier postoperative recovery. We have a robotically assisted platform for minimally invasive surgery that the surgeon operates from a separate console interface that mimics the conditions of open surgery (and yes, akin to a video game environment). The robotic arms have much greater dexterity than conventional laparoscopic instruments, greatly increasing their utility. There is currently discussion of using this technique in the United States as a means to do thyroid surgery from a "transaxillary approach" beginning under the arm rather than through an incision in the neck. Surgical technologies, like other therapeutic approaches, are evaluated for their efficacy in clinical trials.

(Photo: R. Baer)

Marybeth Hughes, M.D. *(left)* and colleagues in CCR's Surgery Branch work continuously to redefine the scope of technologies used in surgical oncology.

Surgically Targeting Liver Disease

The pinnacle of clinical research must be the Phase III multicenter randomized control trial (RCT). Such a trial is the culmination of years, sometimes decades, of research and development, and it is the moment when you can finally prove whether all of the hypotheses, animal data, and encouraging results of earlier human trials have really succeeded in producing a better treatment.

Our branch has been studying ocular melanoma and its metastasis to the liver for some time. Our patients have been diagnosed from their twenties to their seventies, and their prognoses are not good. Fortunately, the disease is rare—ocular melanoma affects 2,000 cases per year with only a fraction developing liver metastases. Unfortunately for the affected few, ocular melanoma is another example of an orphan disease cancer with the associated difficulties in conducting research directed towards cures. The current best effective treatment for these liver metastases leads to survival rates of only 4–6 months.

As a result, some seemingly extreme measures have been used to treat this disease. In the past, we have used a

technique known as open isolated hepatic perfusion to surgically isolate all of the blood vessels in the liver so that we could deliver very high doses of the chemotherapeutic agent melphalan for 60 minutes on the operating table without damaging other more sensitive organs in the process.

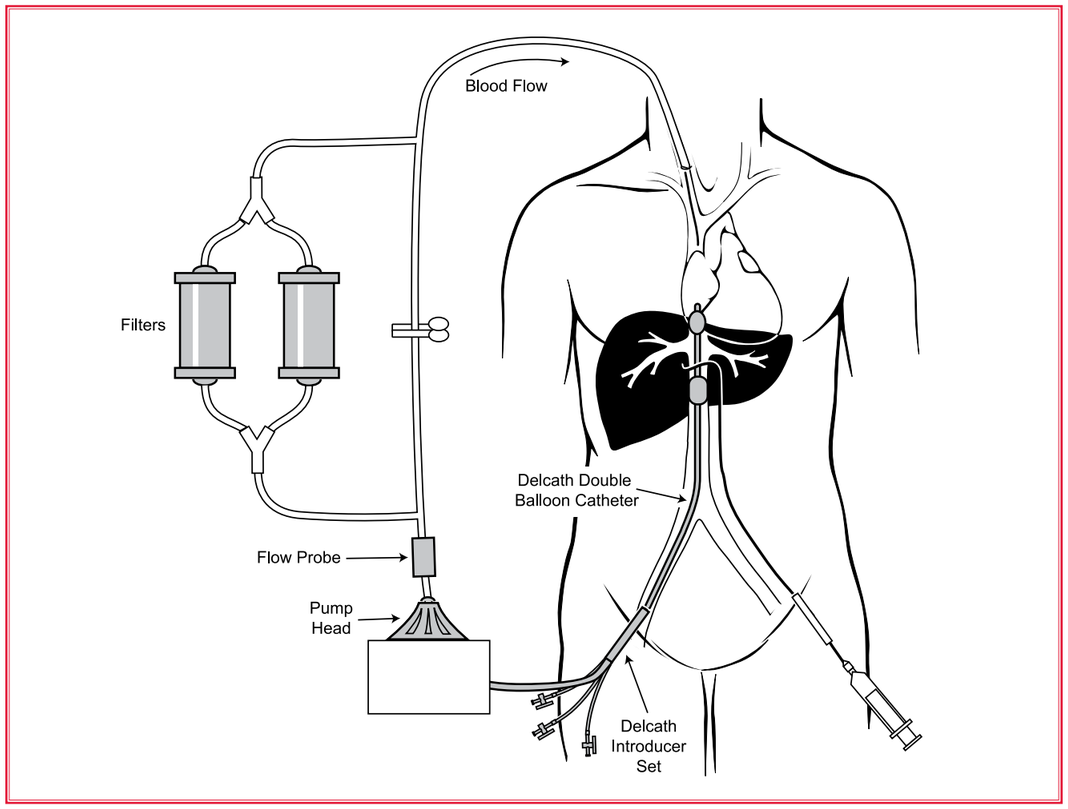
I am currently coordinating a Phase III RCT to treat these patients with peripheral hepatic perfusion in a dozen centers across the country. The goal is to once again deliver melphalan directly to the liver but to do so in a much less invasive manner. Instead of opening up the abdomen to get to the liver, catheters are threaded through the hepatic artery from small incisions in the groin to deliver the drug. Catheters above and below are also inserted to suck out the melphalan-laden blood, which is then filtered before being returned to the body. Each treatment takes from 1–4 hours, and patients can have up to six treatments, depending on how they are responding, particularly to the toxic side effects of the drug.

Advancing surgical technology has refined this macroscopic approach to targeted drug delivery from its much cruder earlier form. We have initiated the use of these techniques to treat other types of tumors, and we hope to use them, in time, to deliver more specific therapeutic agents.

Developing Immunotherapies

When I first joined CCR as a Surgical Oncology Fellow, I was very lucky to have the optimal experience of combining clinical work and research. I joined a project to develop immunotherapies for cancer, which put me exactly in the right place at the right time to participate in translational research at its finest. It was almost a fairy tale of clinical research—to make a novel scientific discovery that goes to the clinic over the course of just a few years. Although I am no longer involved in the laboratory work to develop these therapies, I am still actively involved in testing them in patients and working with my colleagues in the laboratory to further develop them.

The broad concept of immunotherapy is to help the immune system to do its job, namely, to clear out cells that are "bad," whether as a result of infection or disease. Normally, this purging is done by a special class of white blood cells, T lymphocytes, which express receptors (T cell receptors or TCRs) that recognize aberrant proteins on defective cells and then activate the immune cascade that ultimately destroys them. In the case of cancer, the problem for the immune system is to identify the cancerous cells that, in turn, are continuously evolving to evade detection. Certain cancers do display characteristic proteins (antigens) on their cell surface that T lymphocytes are able to recognize, and we can find these T lymphocytes in tumors. However, they are not always present or available in sufficient numbers to mount an effective defense.



(Image: M. Hughes, CCR)

Instead of opening up the abdomen to get to the liver, catheters deliver the drug. They are threaded through the hepatic artery from small incisions in the groin. Catheters above and below are also inserted to suck out the drug-laden blood, which is then filtered before being returned to the body.

In the project I worked on as a Fellow, we asked whether we could introduce a gene that coded for a TCR recognizing a tumor-specific antigen associated with melanoma, MART-1, into a patients' circulating T-lymphocytes to boost the natural immune response. To do this, we isolated lymphocytes from two patient's blood and grew the cells in a dish where we could introduce the TCR gene. Once we were sure that the T-lymphocytes were expressing the TCR, we put them back in the patients and monitored the results. We found that the genetically altered T-lymphocytes remained in circulation for over a year after they were introduced and that the tumors had regressed. The results were published in the journal *Science* in 2006, and we are still working on improving the conditions for this type of adoptive immunotransfer, both for melanoma and for other forms of cancer for which tumor-associated antigens are identified.

There are several other methods for boosting the immune response to cancers that we are actively studying. Adoptive immunotransfer has, thus far, had greater success when we are able to find T-lymphocytes that have infiltrated the tumor already (tumor-infiltrating lymphocytes or TILs). We surgically remove the tumors and grow the cells that have made their way into the tumor in large numbers. Then we give a chemotherapeutic agent to reduce the background immune response and introduce the army of TIL-derived cells into the patient. In melanoma patients, we have had approximately a 50 percent success rate with this approach, and we are currently working to optimize this process through the use of immune modulators like cytokines.

> Like many other medical specialties, surgical oncology is continuously redefining its scope as well as its technology.

We know that introducing cytokines like IL-2 in high doses or inhibitors such as anti-CTLA4 that take the brakes off the immune system can be effective in fighting tumors. However, many of these interventions have resulting toxicities in which the immune system becomes too active and causes problems with autoimmunity. Increasingly, we are realizing that the immune system must achieve a perfect balance in deciding what to attack and that our efforts to circumvent this balance for therapeutic purposes may look promising in the laboratory but have unforeseen consequences in the clinic. For instance, we do not really understand the tumor microenvironment well enough to know how it is responding to our manipulations—it seems clear that there are mechanisms that can turn off all of the switches that we are trying to turn on. Patient-to-patient variability, both in terms of the cancer and the immune system, also makes it hard to tease out the responses we would like to see. To move this kind of research forward really requires continual interaction between lab and clinic to understand what is working and why.

> I believe that cancer immunology holds a very special promise for the future of cancer therapies.

Looking Ahead

Perhaps because it is where I began my work at the NIH, I believe that cancer immunology holds a very special promise for the future of cancer therapies. If you think about the kind of damage that our cells sustain over the course of a normal lifetime, the surprising thing is that only one in four of us is diagnosed with cancer. The role of the immune system in resisting cancer normally must be substantial, and we must be able to tap those normal mechanisms when they fail.

Even in the molecular age, however, there is still a strong role for surgical innovation in the treatment of cancer. We are, of course, learning an enormous amount on the molecular and genetic levels about different types of cancers, and I look forward to the day when we can translate that information into diagnostics and treatments for rare cancers. Like many other medical specialities, surgical oncology is continuously redefining its scope as well as its technology. My work brings me into diagnosis in the form of biopsies, treatment in the form of surgical intervention, and research in the form of developing new biologic-based therapies and new techniques to deliver them.

To learn more about Dr. Hughes's *research, please visit her CCR Web site at http://ccr.cancer.gov/staff/staff.asp?profileid=6156.*

To learn more about the Surgery Branch at CCR, please visit http://ccr.cancer.gov/labs/lab.asp?labid=93.

Where Is the Tumor?

(Photo: Courtesy of B. Hogerty)

Marybeth Hughes, M.D., treated Brandon Hogerty's insulinoma—a rare type of endocrine tumor—at the CCR Surgery Branch. He now looks forward to returning to the university.

To say that Brandon Hogerty owes his life to a social networking site would be a definite exaggeration, but there is nonetheless a grain of truth to it. A few months into his first semester at James Madison University, his friends noticed that he had not emerged from his room in a couple of days, but they thought maybe he was just upset about something. Then a friend from home tried to reach him, and when she could not, she chatted about her concern to mutual friends at the university on Facebook. They found Brandon in bed, unable to move, and phoned for an ambulance.

Brandon's blood level sugars had dropped to zero, and he had suffered a hypoglycemia-induced seizure. "They couldn't get a glucose reading," he was later told.

As many incoming freshmen do, Brandon had come down with a common virus a few days earlier and had not felt like eating anything. Fasting naturally lowers glucose levels, but the fact that his had gone to zero meant that something was seriously wrong. The local hospital was able to stabilize him but could not diagnose the underlying metabolic problem. Doctors back home in Richmond, Va., concluded that Brandon must have an insulinoma—a rare type of endocrine tumor that secretes insulin. Insulin is normally released in response to rising blood sugar levels, but when it is secreted continuously from a tumor, it can easily mop up all of the available blood sugar. There was only one problem—they could not find it.

That is when Brandon was referred to NCI where he underwent a series of diagnostic tests. Marybeth Hughes, M.D., and her colleagues were also unable to find the tumor through conventional imaging methods. However, they were eventually able to localize the tumor to the head of the pancreas by doing intra-arterial calcium stimulation tests—infusing calcium into the arteries feeding the pancreas to induce insulin release and track its source.

Eight months elapsed from the episode of hypoglycemia in college to removal of the tumor. But Brandon and his family had suspected something was amiss even earlier. "For like a year before, I'd start getting the symptoms of hypoglycemia—I didn't know what it was—I just knew I had to eat right away." And his parents noticed that sometimes he would just tune out and "act like a zombie." Because he did not know how long the illness would last, Brandon withdrew from James Madison in late January and, this July, enrolled in community college. He plans to get an associate degree in Business before reapplying to the university.

The doctors say that cases like these are very rare in people as young as Brandon, and so they do not know for sure what the long-term consequences could be. But like any teenager with goals ahead of him, Brandon is happy to be healthy. "I was worried there would be huge scars," he said. "But they appear to be healing nicely."

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